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CLINICAL AND EXPERIMENTAL

AN ATTEMPT TO FIND AN ANTIPEPTIC ENZYME IN THE BLOOD*

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MANY clinicians regard peptic ulcer as a local digestive process in the stomach or duodenum. That the stomach does not digest itself is a physiologic phenomenon for which there is little or no explanation. It seems logical, therefore, that any data that might explain why the stomach does not digest itself might also throw some light on the etiology of peptic ulcer.

Digestion in the stomach and duodenum, proximal to the ampulla of Vater, is due to the action of pepsin and hydrochloric acid. If peptic ulcer is a local digestion (autodigestion) of the stomach or duodenum by its own digestive juices, it follows that peptic ulcer may be in some way related to an abnormal pepsin hydrochloric acid mixture, or there may be something abnormal in the wall of the stomach and duodenum that renders these tissues particularly susceptible to the action of the digestive juices in such cases.

The first possibility mentioned, namely, an abnormal pepsin hydrochloric mixture in stomachs with ulcer, has been the subject of much investigation. The textbooks emphasize a high free hydrochloric acid content in ulcer cases, and it is the general inference that in some way the high hydrochloric acid content is responsible for the ulcer. However, it is not uncommon to find patients suffering from ulcer in whose gastric contents free hydrochloric acid is not found. Such findings, even in a few cases, suggest very strongly that the high free hydrochloric acid is probably not the only factor concerned in the formation of peptic ulcer.

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The pepsin factor in the pepsin hydrochloric acid mixture is one that scarcely lends itself to investigation at this time. It is true that patients with ulcer may have a particularly active pepsin thrown out by the gastric mucosa. However, in the first place, we do not know what pepsin is; it has never been isolated as a chemical entity. We have reason to believe that it is a colloid; we know that it is an enzyme, which, in the presence of free acid brings about proteolytic activity, but our specific knowledge concerning it is deficient. It follows, therefore, that until we know the nature of normal pepsin we cannot possibly point out an abnormal pepsin and assign to such an abnormal product any etiologic relationship to peptic ulcer.

The second possibility is whether or not there is something which renders the stomach and duodenum particularly susceptible to the action of their own digestive juices in ulcer cases. What causes the stomach to digest meat that is ingested as food, and yet does not normally affect the wall of the stomach itself? We are, it seems, considering a problem of much the same nature as the old question of how would it be possible to have a container for a universal solvent. Yet here, there is a solution for that old riddle. The digestive juices will dissolve any meat exposed to them, and yet will not dissolve the walls of the stomach, which are apparently physically and chemically identical to the substance being dissolved. However, the meat ingested as food differs from the flesh of the wall of the stomach, at least in one respect: the one has no blood supply; the other has an intact blood supply which results in a continual bathing of all of its cells with blood and lymph. An excellent experimental review of this question has recently been made by Draagstedt and Vaughn. Is it possible that the circulating blood and lymph protect the stomach and duodenal mucosa from the action of the digestive juices; and is it a lack of such protective substances in the blood stream that results in digestion and ulcer formation in certain cases? In other words, is there a substance in the circulating blood which inhibits peptic activity; and if so, is the lack of, or absence of that substance in any way related to the formation of peptic ulcer? This research was undertaken in an attempt to answer these questions.

REVIEW OF LITERATURE

Since Weinland's work, discussions have appeared in the literature concerning a substance in the blood stream, which is considered to be of the nature of an antienzyme; it is referred to as antipepsin.

Weinland, in working with ascaris worms, was able to extract from them a substance which had a definite pepsin inhibitory effect. He concluded, therefore, that the reason these worms are not digested in the stomach, but are able to pass on and live in the intestine, is because of this pepsin inhibitory substance in their bodies. He suggested further that the reason the stomach does not normally digest itself is because there is probably a similar pepsin inhibitory substance in its walls, or in the circulating blood and lymph.

Sachs injected geese intraperitoneally with increasing doses of pepsin over a period of two and one-half months. He was able to demonstrate the presence of an antipepsin ferment in the blood of these geese. He found, by his method of gelatin liquefaction, that it took twenty times the amount of

pepsin to digest (render fluid) gelatin in the presence of goose blood injected with pepsin, as in the presence of normal goose blood. He explained his findings as due to the development of an antipepsin (a pepsin inhibitory substance) in the blood of geese injected with pepsin, and calls attention to the similarity of this antipepsin to antitoxins, a similarity noted also by Kolmer and Wells.

Oguro likewise investigated the presence of antipepsin in the serums of horse and man. He prefers the use of fibrin carmine in his demonstration, and considers the liberation of the carmine in his series of tubes as evidence of digestion.

Bayliss, however, is unable to demonstrate antienzymes and sums up his opinion as follows: "There is considerable doubt whether true antienzymes exist. Some of the effects described as being due to them are to be accounted for by changes in hydrogen-ion concentration, others by the absorption of the enzymes by colloids."

Hamburger attributes many of the reports of antipepsin as probably due to the inhibitory effect of sodium chloride used in the solutions and manipulations, and he demonstrates that physiologic sodium chloride solution can definitely inhibit peptic activity.

Kolmer sums up the present status of the antiferment question as follows: "According to some investigators antiferments are to be found in large amounts in all normal sera and are probably vitally concerned in life processes in preventing autodigestion. That they may be artificially stimulated is disputed. They certainly never attain the amounts as antitoxins. Various antiferments have been produced according to many investigators but these observations have not been generally confirmed."

METHODS

An investigation was first made of the various methods described in the literature for demonstrating antipepsin. Specimens of blood from normal persons, ulcer-bearing patients, and patients suffering from other diseases, but in whom the diagnosis of peptic ulcer had been eliminated, were used. Blood from normal dogs and from dogs in whom peptic ulcer had been produced by means of Mann's method of draining the alkaline secretion away from the pylorus were also used. The methods investigated depend primarily on a measuring of peptic activity. The antipepsin is estimated by its inhibitory effect on peptic activity.

Liberation of Dye and Protein.—The method employed by Oguro and Hamburger was tried. They use a protein, fibrin, in which carmine is incorporated. A series of tubes is set up, containing increasing amounts of pepsin, constant amounts of hydrochloric acid, and constant amounts of the serums whose antipeptic properties are to be studied. Then flakes of fibrin-carmine are added and the tubes incubated. After incubation, the tubes containing the larger amounts of pepsin are red, due to the liberation of the carmine on digestion of the fibrin; the tubes containing the smaller amounts of pepsin are not so colored because there is no digestion, and hence no liberation of carmine. The reason, therefore, that the pepsin in the colorless tubes had

been bound is due to the presence of antipepsin in the additional serum. The amount of antipepsin present in the serum is then estimated by noting the greatest amount of pepsin that will not produce liberation of the dye in the presence of that serum.

This method was given a thorough trial. Fibrin was prepared by drawing blood in oxalate solution and centrifugalizing out the red cells. The serum or fibrin was then recalcified and clotted, then cut into small bits, the size of a split pea, and soaked in a 0.5 per cent carmine solution. After twenty-four to thirty-six hours the fibrin was thoroughly impregnated with the dye. The particles were then washed with running water for from twenty-four to thirty-six hours. The serum pepsin hydrochloric mixtures were set up in tubes as described by Oguro and Hamburger. The gradation between the colorless tubes and the red tubes was so gradual as not to give any definite idea of an end point. Further, it was not uncommon to get a liberation of the dye in control tubes in which we had added simply hydrochloric acid and no pepsin. This method, therefore, was not applicable to the problem.

Gelatin Liquefaction.—The gelatin liquefaction method of Sachs was investigated. A series of tubes of gelatin solutions from 3 to 18 per cent were set up. These were liquefied and then allowed to stand until the thermometer within the tube registered the same temperature as a thermometer on the work table, thus making sure that the tubes had come down to room temperature. It was noted that when both thermometers registered 22.2° C., the 9 per cent gelatin was solid; the 7.2 per cent gelatin was solid but quivered; the gelatin solutions less than 7 per cent in strength were all fluid. This rack was allowed to stand three hours longer, and then the solutions were observed again. The two thermometers still registered 22.2° C., but at this time the 6 and 5 per cent solutions which were fluid on the first observation had become solid. It seemed, therefore, that gelatin was not suitable as an indicator of digestion, as its state of fluidity or solidity seemed to depend not only on the temperature, but also on other factors. Since it was not known whether a solution of undigested gelatin of a given per cent and at a given temperature would jell or be fluid, the state of digestion could certainly not be estimated by its fluid or solid state.

Mett Tube Principle.—An attempt was made at this point to use the old Mett tube principle of measuring digestion. It was apparent that for this problem Mett tubes would not be sufficiently accurate unless they were more accurately made and standardized than those usually employed in experiments on digestion. Accordingly the Mett tube principle was used, but standardized in a different manner, namely, a measured quantity (0.05 c.c.) of coagulable protein was placed in the bottom of Wassermann tubes. Blood serum from various sources and egg white were used. This protein was carefully shaken to the bottom of the tube, and then coagulated by placing the rack of tubes in boiling water for three minutes. After coagulation, increasing amounts of pepsin solution (constant quantities of serum being examined) and hydrochloric acid were added. The tubes, after digesting in the incubator, were turned upside down. It was hoped that complete digestion would be shown by absence of the coagulated serum, or egg white, in the

bottom of the tube, and incomplete or no digestion by the presence of this coagulated protein in the tube. However, this method likewise proved inaccurate, inasmuch as very often the undigested protein could be seen to slip away from the bottom of the tube. The digestion mixture was turbid, and the question continually arose as to whether or not the turbid tubes near the end point were not due to undigested protein that had fallen away from the bottom of the tubes. Further, although the quantity of protein was constant, within limits of experimental error, the surface areas were not constant and this is a possible cause for error.

Physical and Chemical Methods.—An investigation was made into the physical and chemical methods described in the literature for measuring digestion, with the view that, once the proper index of digestion was found, it could readily be applied to the measuring of an antipepsin by the antipepsin's inhibitory effect on peptic digestion, as shown by the given index of digestion. At our disposal were: (1) rotation of polarized light, 10; (2) changes in electrical conductivity, 11; (3) changes in index of refraction of light, and (4) determination of nonprotein nitrogen, 12.

Therefore, a series of tubes were set up containing blood serum, 1 c.c.; pepsin, 1 c.c. (1:100 solution), and one-tenth normal hydrochloric acid, 8 c.c. The serums used were from cases of ulcer, normal controls, and dogs injected with pepsin. It was considered unnecessary to add protein for the pepsin to digest, since the serum being examined contained 6 to 8 per cent protein. Polariscopic and refractometer readings were taken on these solutions, before and after digestion. The differences in readings before and after digestion were so small, if any, that they were entirely within the limits of error of the apparatus.

Similar tubes were set up, as in the preceding experiment, and the nonprotein nitrogen was determined before and after digestion. There was no constant difference in the nonprotein nitrogen, before and after digestion, that designated ulcer or normal bloods in the group.

DISCUSSION

Throughout this work, as in the beginning, the question continually arose as to whether or not the substance being sought did, or did not actually exist. The question is still an open one. The methods described in the literature as demonstrating an antipepsin were used, and were found to be either not sufficiently accurate for the problem, or entirely incapable of application. The technical difficulties are numerous. There are many factors which have to be constantly controlled. In the first place aseptic technic is desirable, although difficult to carry out. The pepsin solution must be sterile, and kept sterile by passage through a Berkefeld filter. The pepsin used was regularly contaminated by a Gram-positive proteolytic bacillus, not unlike B. subtilis, but the organism failed to give the characteristic sugar reactions of B. subtilis. The conditions of digestion must be accurately controlled. The hydrogen-ion concentration plays an important part. It was practically impossible to keep this factor constant, except within rather wide limits, owing to the fact that

the digestion reaction must necessarily go on in the presence of the examined serum. These serums all vary in their hydrogen-ion concentration.

There are other factors such as, (1) temperature of digestion mixture, (2) length of time digestion is permitted to proceed, (3) total volume of the digestion mixture, in order to dilute digestion products which inhibit digestion, (4) the strength of the pepsin solution, and (5) the length of time during which the pepsin and serum are allowed to remain in contact with each other before adding the hydrochloric acid. All of these factors play a part, and it may be that an ideal combination of all of them will lead to findings other than our own.

The paramount difficulty is the method of measuring digestion. The methods of liberation of a dye from protein and of gelatin liquefaction, in our hands, have not proved applicable to the problem. Measurement of changes in the physical properties of the digestion are of no avail. The measurement of nonprotein nitrogen as an index of digestion seemed promising, but we were at once confronted with the choice of a protein precipitant. It is possible that the precipitants used, that is, sodium tungstate, trichloracetic acid, and sulphosalicylic acid, carried with them certain digestion products which resulted in our variable findings.

At any rate, it is probable that the antipepsin spoken of in the literature has not been demonstrated. The methods described in the literature as applicable to their demonstration do not prove the presence of such a substance, and no doubt an antipepsin will be proved to be present or absent only when more accurate methods for its detection have been evolved.

SUMMARY

In view of the fact that peptic ulcer is considered a local autodigestion of the stomach, an investigation was made of the protective antienzyme theory of gastric protection. This theory, if it explains lack of autodigestion of the stomach, should likewise be applicable to the etiology of peptic ulcer. The methods described in the literature as demonstrating an antipepsin were used and found to be inaccurate. These methods neither proved nor disproved the presence or absence of such a substance. Antipepsin may or may not exist, and it may or may not have a bearing on the etiology of peptic ulcer. More accurate methods must be devised before this question of the relationship between antipepsin and peptic ulcer can be answered.

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A STUDY OF INSULIN ACTION*

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SINCE the introduction of insulin there has developed a very comprehensive literature covering its preparation and clinical application. However, little has developed as to how insulin enables the individual to utilize sugar. This latter point has been recently emphasized in a brief report made by Banting[1] on the "Pharmacologic Action of Insulin."

Previous studies, attempting to determine the mechanism of blood-sugar reduction by insulin, have been along four general lines.

First: The action of insulin on the blood—Eadie, Macleod, and Noble.[2]

Second: The action of insulin in the blood stream with the presence of extracts of glands of internal and external secretions—*a.* adrenalin: Ross and Davis[3] and Banting, Best, Collip, Macleod and Noble[4]; *b.* pituitary extract: Burns[5] and Olmstead and Logan[6]; *c.* parathyroid extract: Winter and Smith[7] and Forrest[8]; *d.* trypsin: Epstein, Rosenthal et al.[9]

Third: The action of insulin following the removal of viscera—*a.* thyroidectomy: Bodansky,[10] Ducheneau,[11] and Burns and Marks[12]; *b.* adrenalectomy: Stewart and Rogoff[13]; *c.* hepatectomy: Mann and Magath[14]; *d.* decapitated and eviscerated cats: Burns and Dale.[15]

Fourth: The action of insulin in perfusion experiments—*a.* isolated heart: Maclean and Smedley,[16] Knowlton and Starling,[17] Patterson and Starling,[18] Clarke,[19] Hepburn and Latchford,[20] Burns and Dale.[15]

Although these methods have not been successful in determining the exact mechanism of insulin action, there have been discovered measures whereby the lowering of blood sugar by insulin may be either completely neutralized or greatly enhanced.

Apparently the mechanism of insulin action must be considered as being of twofold: its *primary* action and its *secondary* action. The primary action covers only the mechanism of blood-sugar reduction while the secondary action is observed in the convulsions which accompany the hypoglycemia of excessive doses of insulin. In the present work we have attempted to study only the primary action, as the secondary action has been carefully studied by Olmstead and Logan[6] and Cannon, McIver and Bliss.[21]

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In reviewing the literature on insulin, we were impressed with the possibility that the rôle of the nerve mechanism in insulin action had not been fully considered. We have therefore made a study of this phase of the problem, thinking it might yield some additional information on the subject. Our object has been to stimulate or paralyze portions of the autonomic and central nervous systems and follow such procedures with insulin administration and blood-sugar determinations.

METHOD

We have used only unanesthetized animals as it is a well-established fact that anesthesia has a marked effect on the metabolism of body sugar. In our studies involving the destruction of the central nervous system, we gave preliminary ether anesthesia but discontinued it as soon as possible. The insulin used was that of Eli Lilly Co., and all administrations were made intravenously, the dose usually being four units per kilo in both the rabbit and dog. Blood samples were taken before the administration of insulin and usually at periods of one-half hour and one and one-half hours following. The blood was obtained from the marginal ear veins in the rabbit (2 c.c. samples) and from the femoral vein in the dog (5 c.c. samples). The blood-sugar determinations were made according to the method of Folin and Wu.[22]

STUDIES ON THE SYMPATHETIC SYSTEM

It has been demonstrated by Banting, Best, Collip, Macleod, and Noble[4] that there exists an antagonism between adrenalin and insulin in their action on blood sugar. This observation has been repeated by other workers and in order to standardize our technic, we have repeated similar experiments using both the dog and the rabbit. Two of such experiments are briefly summarized in Table I.

TABLE I

DOG 1. MALE, 15 KILO. NOT FED IN PREVIOUS TWENTY-FOUR HOURS

		TIME	BLOOD SUGAR
	Normal Blood Sugar		94.3
	Insulin 60 units	7.53	
	Blood Sugar	8.32	66.2
	Blood Sugar	9.15	53.7
	2 c.c. Adrenalin (1-10000)	9.22	
	Blood Sugar		62.5
RABBIT 1.	2 KILO.	9.57	117.6
	Insulin 8 units	10.12	
	Blood Sugar	10.50	78.8
	Blood Sugar	11.42	76.1
	1 c.c. Adrenalin (1-10000)	11.45	
	Blood Sugar	11.55	92.9

The power of adrenalin to thus counteract the hypoglycemia of insulin suggests the possibility that the two drugs may be antagonistic physiologically. Therefore, in order to test the rôle of the sympathetic system in the production of hypoglycemia by insulin, we have attempted to paralyze the sympathetic ganglion by use of nicotine and lobeline and follow their administration by insulin. Table II briefly outlines the results.

TABLE II

RABBIT 5. 3.64 KILO

	TIME	BLOOD SUGAR
0.5 c.c. Nicotine 0.5% (nearly died)	3.21	
0.25 c.c. Nicotine 0.5%	3.30	
Blood Sugar	3.32	150.4
Insulin 15 units	3.37	
Blood Sugar	4.10	44.5
Blood Sugar	5.10	47.8
DOG 5. 17 KILO. NOT FED IN PREVIOUS TWENTY-FOUR HOURS		
Blood Sugar	9.35	112.2
Lobeline 0.5 c.c. (0.5 mg./c.c.)	9.40	
Lobeline 1.0 (0.5 mg./c.c.)	9.45	
Insulin 70 units	9.52	
Lobeline 1.5 c.c. (0.5 mg./c.c.)	9.55	(definite reaction)
Blood Sugar	10.27	64.5
Blood Sugar	11.22	58.0

From the results shown in Table II it is suggested that the sympathetic system is not primarily involved in the production of hypoglycemia by insulin. We have confirmed these results with varying doses of lobeline and nicotine in combination with atropine and partial destruction on the central nervous system.

STUDIES ON THE PARASYMPATHETIC SYSTEM

Garrelton and Santenoise[23] have noted that insulin is a powerful excitant of the vagus, the oculocardiac reflex being augmented, the heart and respiration slowed, and the blood pressure falls. The susceptibility to shock increases. They found that such a picture was independent of blood-sugar decrease, as previous injections of glucose did not influence the results. They suggest that albumin in the insulin may modify the results and that certain hypovagotonic animals do not respond. Houssay and Lewis[24] have observed that the blood-sugar increase which accompanies morphine may be hindered by section of the splanchnics, but comes into play again if the vagi are cut. We have observed several animals which have received atropine before insulin and Table III expresses the results.

TABLE III

DOG 4. FEMALE, 17 KILO. NOT FED IN PREVIOUS TWENTY-ONE HOURS

	TIME	BLOOD SUGAR
Blood Sugar	9.20	84.8
Atropine 1 mg.	9.29	
Atropine 1 mg.	9.33	
Blood Sugar	9.38	84.1
Insulin 68 units	9.42	
Blood Sugar	10.15	61.7
Blood Sugar	11.30	57.4
RABBIT 5. (PREVIOUSLY REPORTED.) 3.64 KILO.		
Atropine 5 mg.	3.15	
Atropine 7.5 mg.	3.17	
Nicotine		
Blood Sugar	3.32	150.4
Insulin 15 units	3.37	
Blood Sugar	4.10	44.5
Blood Sugar	5.10	47.8

We have confirmed these results by using atropine with other drugs and from the findings, it appears that insulin acts as effectively after the animal has received large doses of atropine.

PITUITARY EXTRACT

Burns,[5] in his splendid studies on insulin and pituitrin, has demonstrated that subcutaneous injections of the extract of the posterior lobe of the pituitary gland given simultaneously with injections of insulin, diminished or abolished the fall of blood sugar normally produced by the insulin. He has further shown that pituitary extract removes the symptoms of hypoglycemia convulsions causing a rapid elevation of blood sugar. We have been able to confirm his observations as illustrated in Table IV.

TABLE IV

DOG 2. 15 KILO. NOT FED IN PREVIOUS EIGHTEEN HOURS

	TIME	BLOOD SUGAR
Blood Sugar	9.25	74.7
Insulin 60 units	9.30	
Blood Sugar	10.5	33.7
Blood Sugar	11.10	33.2
Pituitrin 1 c.c.	11.16	
Blood Sugar	11.32	58.5
RABBIT 2. 2.27 KILO		
Blood Sugar	9.45	108.1
Insulin 9 units	9.51	
Blood Sugar	10.22	44.8
Blood Sugar	11.27	43.7
Pituitrin 0.3 c.c.	11.29	
Blood Sugar	11.40	81.3

It is impossible to explain how adrenalin and pituitrin acting on any single known peripheral nerve mechanism can counteract the hypoglycemic action of insulin. Burns has called attention to the fact that although both adrenalin and pituitrin produce a hyperglycemia, adrenalin produces a greater and more prolonged hyperglycemia and the antagonistic action of pituitrin to insulin hypoglycemia is more extensive, more persistent, and more rapid in its action.

STUDIES ON CENTRAL NERVOUS SYSTEM

The observations of Cushing[25] and his collaborators serve to show that there is a definite relation between carbohydrate metabolism and the pituitary body. Clinically and experimentally they noted that hypophyseal deficiency was accompanied by "an acquired high tolerance for sugars, with the resultant accumulation of fat." Borchardt[26] found that infundibular extract caused a rise in blood sugar. However, such an increase was denied by Franchini.[27] Later, Cushing showed that the intravenous administration of the extract was "almost without exception" accompanied by hyperglycemia.

In attempting to study insulin convulsions in decerebrated cats Olmstead and Logan were unable to cause a lowering of blood sugar in the decerebrated animals to the level necessary to produce convulsions in the rabbit. They then attempted to determine whether such a high blood sugar in the decere-

brated cat was not attributable to the action of the pituitary body. After considerable work on decerebrated cats with the pituitary body intact, and with the pituitary body removed, they concluded that "decerebrated cats with the pituitary body intact maintain a high blood-sugar level which is not materially reduced by insulin." Analysis of their results indicate that both the animals with the pituitary body intact and with the pituitary removed responded to the administration of insulin, the amount of blood-sugar lowering being essentially the same in the two conditions. However, in the animals with the intact pituitary the blood sugar was at a higher level at the time of the insulin administration and therefore the resulting blood sugar did not reach so low a level.

Burns and Dale[15] have recently published experimental results which have a very definite relation to this phase of our work. They observed that in the decapitated and eviscerated cat, with constant infusion of dextrose, insulin produces the characteristic fall of blood sugar.

In our studies involving the central nervous system we have attempted to avoid asphyxiation, for Yamakani[28] has demonstrated that clamping the trachea of the rabbit would increase the blood sugar from normal, i.e., about 0.120 per cent to 0.225 per cent, such increase occurring in five minutes. We have not attempted any studies which would necessitate the intact respiratory center but instead have used artificial respiration which could be accurately controlled and thereby have avoided any changes which might be caused by respiratory stimulation or depression.

In destroying the central nervous system we have used essentially the technic described by Jackson.[29] We have studied both the action of insulin after destruction of the brain and cord and also with the addition of atropine, lobeline and nicotine. Table V represents the results obtained after pithing an animal's brain and cord.

TABLE V

DOG 12.7 KILO. FED TWENTY-FOUR HOURS PREVIOUSLY

	TIME	BLOOD SUGAR
Blood Sugar (under ether)	11.00	180.1
Pithed	11.07	
Blood Sugar	11.17	192.2
Blood Sugar	11.27	168.5
Insulin 70 units	11.31	
Blood Sugar	12.01	74.1
Blood Sugar	12.18	49.0
Insulin 80 units (Stearn's)	12.21	
Blood Sugar	12.53	63.7

Comment: Blood sugar apparently increased following the second injection of insulin.

In the second animal of this series, we did not only pith brain and cord but in addition gave 4 milligrams of atropine in divided doses and also 10 milligrams of nicotine in divided doses. Fig. 1-*a* is a record obtained from the animal after it had been given atropine, nicotine and a pithed brain and cord. The record was made during the period which insulin action was studied. It will be noted that the animal received two injections of insulin—each representing 100 units, yet there was no reduction of blood sugar but

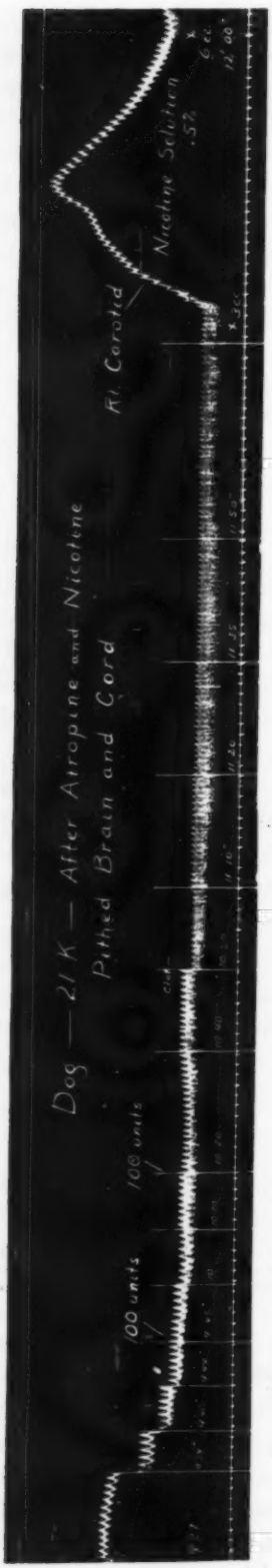
Dog — 21 K — After Atropine and Nicotine
Pithed Brain and Cord
Rt. Carotid
Nicotine Solution
.5%
100 units
100 units

Fig. 1-a.

Dog — 212 K — After Atropine, Lobeline
Pithed Brain and Cord
80 units
100 units
100 units
100 units

Fig. 1-b.

TABLE VI

DOG 21 KILO. NOT FED DURING PREVIOUS THIRTY-SIX HOURS

	TIME	BLOOD SUGAR
Blood Sugar	8.45	97.1
Atropine 4 mg. (Divided doses)	9.00	
Nicotine 2 c.c. 0.5% (Divided)	9.15	
Pithed	9.27	
Blood Sugar	9.35	177.0
Blood Sugar	9.42	200.
Insulin 100 units	9.45	
Blood Sugar	9.55	256.4
Blood Sugar	10.15	285.7
Insulin 100 units	10.18	
Blood Sugar	10.28	317.5
Blood Sugar	10.48	307.7

instead there was a gradual increase. A sample of this same insulin was returned to Eli Lilly and Co., who reported it to be of original potency. It will also be noted that toward the end of the record the animal received a large dose of nicotine which caused a definite blood pressure response. Such a result suggests that the original dose of nicotine was not sufficient to cause

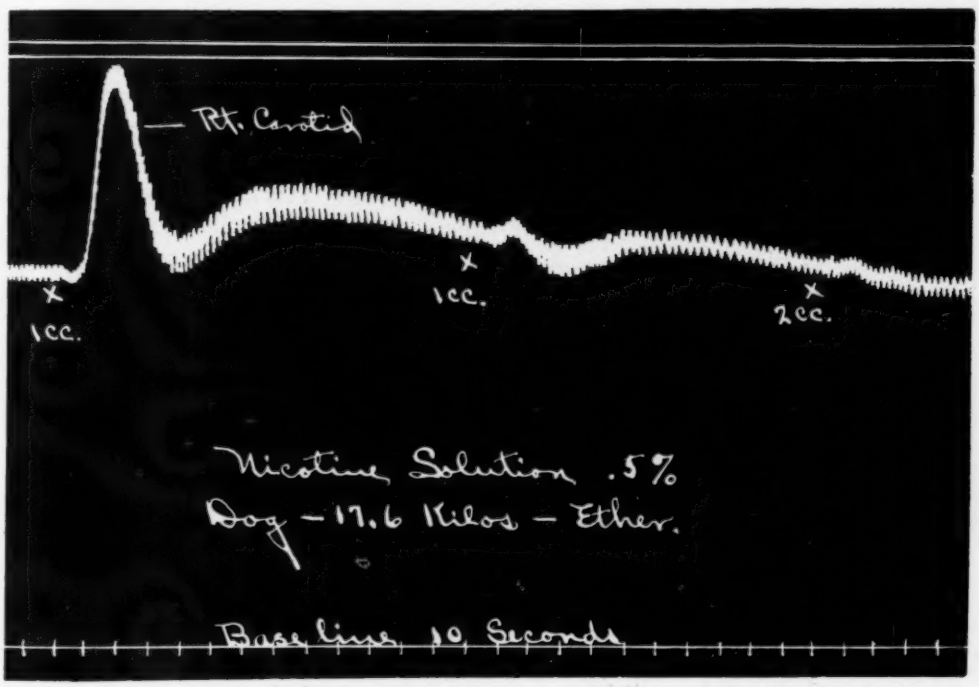

Fig. 2.

paralysis or that its action had worn off during the experiment. Table VI is a summary of procedure and results obtained.

We have repeated the experiment explained in Table VI but using considerably more nicotine. Fig. 2 is the record obtained during the administration of nicotine, the animal having previously received 4 mg. of atropine sulphate. It will be noted that following the first injection of 1 c.c. of nicotine

solution there was a prompt and pronounced rise in blood pressure, the second dose of 1 c.c. of nicotine solution gave little response, while the third injection of 2 c.c. caused practically no change in blood pressure. Following the administration of nicotine the animal was pithed completely, brain and cord. The data presented in Table VII gives a brief record of procedure and results obtained.

TABLE VII

DOG 17.6 KILO. NOT FED DURING PREVIOUS TWENTY-FOUR HOURS

	TIME	BLOOD SUGAR
Blood Sugar (Under Ether)	2.30	123.5
Atropine 1 c.c. (1 mg.)	2.33	
Atropine 1 c.c.	2.35	
Atropine 2 c.c.	2.36	
Nicotine 1 c.c. (0.5%)	2.39	
Nicotine 1 c.c.	2.41	
Nicotine 2 c.c.	2.42	
Blood Sugar	2.44	159.4
Pithed	2.55	
Blood Sugar	3.08	119.8
Repithed	3.11	
Blood Sugar	3.18	111.6
Insulin 100 units	3.25	
Blood Sugar	3.55	84.1
Blood Sugar	4.25	60.6
Blood Sugar	4.55	39.4

It will be observed that during the administration of the atropine and nicotine the blood sugar rose from 123.5 mg. to 159.4 mg., also that this animal, although receiving a much larger dose of nicotine, still responded to insulin administration. These observations suggested to us the possibility that probably nicotine had exerted only a stimulating effect in the previous experiment, and through stimulation of the sympathetic system had left the blood sugar high even in the presence of excess insulin. (We repithed the animal because of some twitching about the mouth which suggested incom-

TABLE VIII

DOG 17 KILO. NOT FED PREVIOUS FORTY-EIGHT HOURS

	TIME	BLOOD SUGAR
Blood Sugar (Under Ether)	10.20	119.1
Blood Sugar (Under Ether)	10.33	137.4
Pithed	10.50	
Atropine 1 mg.	10.52	
Blood Sugar	11.05	196.1
Blood Sugar	11.20	116.3
Blood Sugar	11.35	122.0
Nicotine 2/3 c.c. (0.5%)	11.39	
Blood Sugar	11.45	114.9
Nicotine 0.5 c.c. (0.5%)	11.52	
Insulin 95 units		
Blood Sugar	12.02	111.7
Nicotine 0.5 c.c. (0.5%)	12.05	
Blood Sugar	12.12	95.2
Nicotine 0.5 c.c. (0.5%)	12.15	
Blood Sugar	12.22	87.3
Blood Sugar	12.52	71.4
Artificial respiration discontinued	1.45	

plete pithing. However, we later concluded that such movements were caused by the administration of nicotine.)

Assuming that nicotine in small doses might exert a stimulating effect on the sympathetic system and thereby maintain a high blood sugar, we attempted the following experiment to determine whether such an action was responsible for some of our results. We also desired to observe the variations in blood sugar of an animal pithed brain and cord. The results of our observations are recorded in Table VIII.

On account of the extreme action of the vagus, following the pithing of

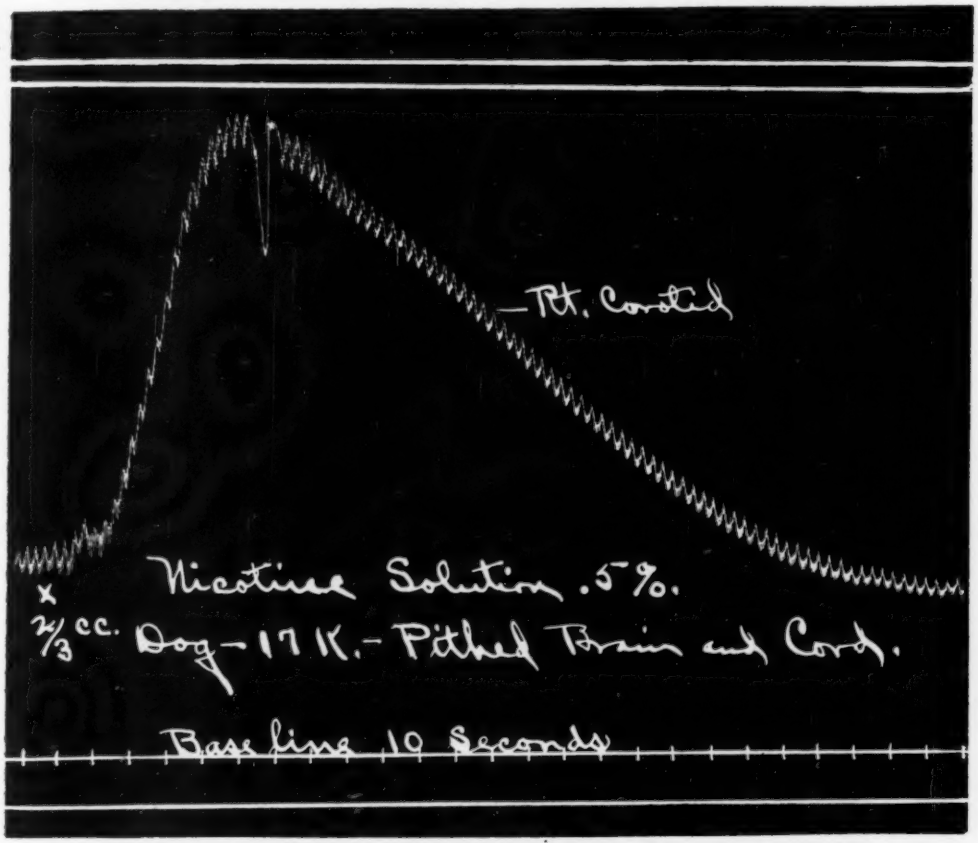

Fig. 3.

the animal, it was thought necessary to give atropine. One milligram was sufficient to free the heart from what appeared to be a dangerous inhibition. The blood sugar rose, following pithing, to 196.1 mg. and during the following thirty minutes appeared to reach a rather constant level of about 122 mg.

After the blood sugar had attained such a constant level the animal received ⅔ c.c. nicotine solution 0.5 per cent. Fig. 3 is presented to show the blood pressure response to the nicotine administration. The blood sugar, following the return of the blood pressure to normal, was essentially the same as before the administration of the nicotine. The animal then received 0.5 c.c. of nicotine solution and simultaneously 95 units of insulin. The blood

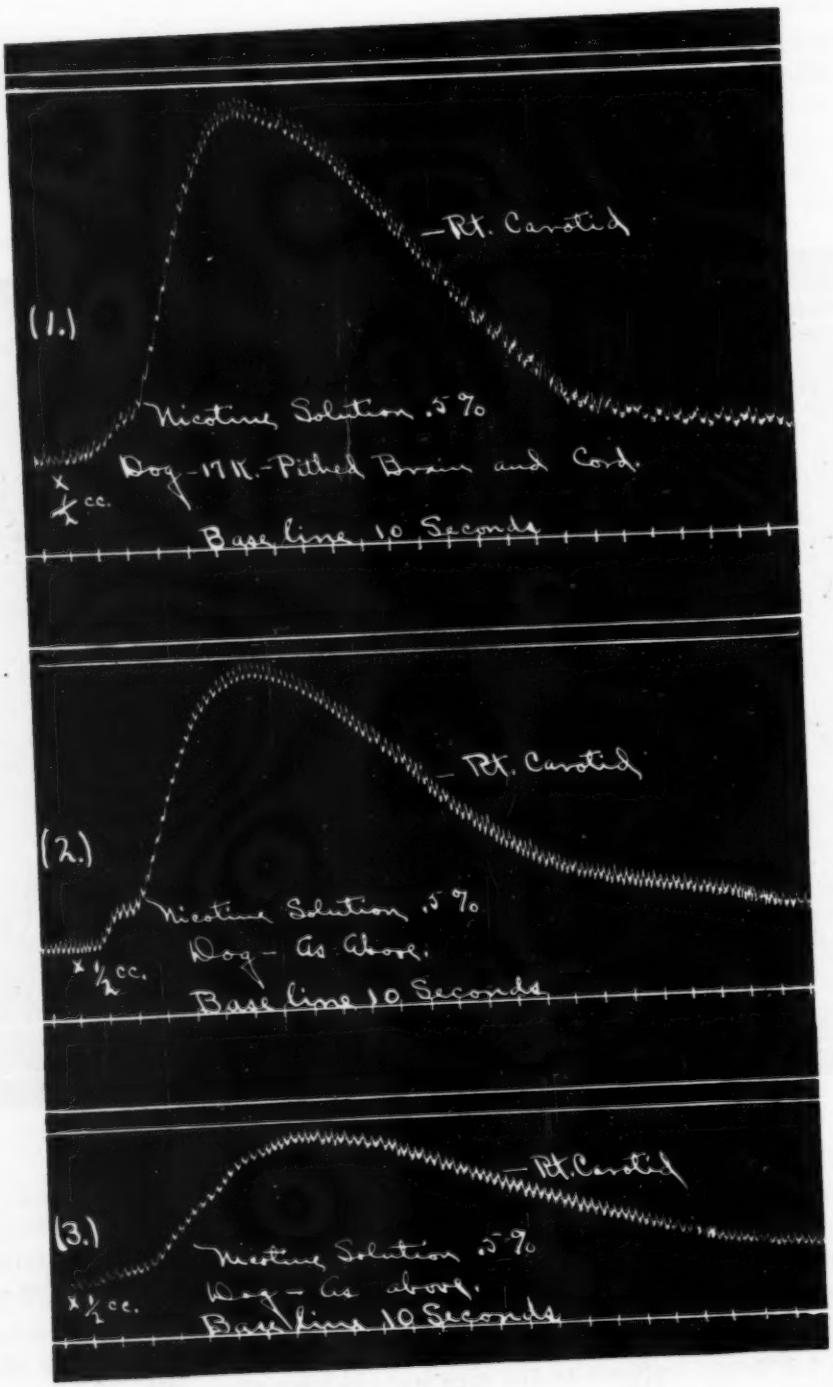
(1.)
Rt. Carotid
Nicotine Solution .5%
Dog-17K.-Pithed Brain and Cord.
x ¼ cc.
Base line 10 Seconds
(2.)
Rt. Carotid
Nicotine Solution .5%
Dog - As above.
x ½ cc.
Base line 10 Seconds
(3.)
Rt. Carotid
Nicotine Solution .5%
Dog - As above.
x ½ cc.
Base line 10 Seconds

Fig. 4.

sugar was determined each ten minutes and 0.5 c.c. nicotine solution was also administered each ten minutes. Fig. 4 shows the result of such administrations—the response becoming less with each injection, yet each produced a definite increase in blood pressure. The results of the blood-sugar determinations accompanying the insulin-nicotine administration are presented in Table VIII.

Table IX is the summary of the data obtained from an animal which had received insulin following the administration of atropine and lobeline. The blood pressure record of the animal is presented in Fig. 1-*b*.

TABLE IX

DOG 21.2 KILO. SPLEEN PREVIOUSLY REMOVED

	TIME	BLOOD SUGAR
Atropine 1 c.c. (1 mg. per c.c.)	3.40	
Lobeline 8 c.c. divided doses (0.5 mg. per c.c.)	3.50	
Pithed brain and cord	4.05	
Blood Sugar	4.10	208.1
Blood Sugar	4.20	208.1
Insulin 80 units	4.22	
Blood Sugar	4.32	246.9
Blood Sugar	4.52	188.7
Blood Sugar	5.52	56.5
Blood Sugar	7.02	33.5
Insulin 100 units	8.18	
Blood Sugar	8.51	100. (Arterial Blood)
Insulin 100 units	9.22	
Insuliln 100 units	10.09	
Insulin 100 units	11.07	
Stopped artificial respiration	11.50	

Comment: Blood sugar rose immediate'y following the second administration of insulin. (The animal received several times the usual toxic dose of insulin without any apparent toxic results.)

CONCLUSIONS

1. Insulin produces blood-sugar lowering after the following procedures: (*a*) the destruction of the central nervous system, (*b*) the administration of atropine in doses sufficiently great to paralyze the parasympathetic endings, and (*c*) the administration of nicotine and lobeline in doses which stimulate and paralyze the sympathetic ganglia.

2. One animal of the series, which was pithed brain and cord and which also received atropine and nicotine, showed a marked increase in blood sugar following the administration of excessive doses of insulin.

3. Small doses of nicotine which stimulate the sympathetic system do not counteract the blood-sugar lowering produced by insulin.

4. Insulin apparently acts independently of the central nervous system, the sympathetic nervous system, and the parasympathetic nervous system.

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FINANCING CLINICAL PATHOLOGY IN THE MODERN HOSPITAL*

By Ward Burdick, M.D., Denver, Colorado

IT is again my privilege, as the representative of the American Society of Clinical Pathologists, to express the appreciation of the courtesy in extending the opportunity to present a subject, in the endeavor to effect a reconciliation in what has probably been one of the most difficult problems, which has confronted the advocates of hospital standardization up to the present time.

It is the purpose of this presentation to represent a composite of the opinions derived from the fellows of the Society as the result of a questionnaire.

The concluding paragraph of the communication presented a year ago before the Congress at Chicago, may be quoted: "In closing may I suggest the appointment of a committee by the American College of Surgeons to cooperate with a similar committee from the American Society of Clinical Pathologists, to formulate a plan of financing the department of clinical pathology in the hospital?" In the absence of action proceeding from this suggestion, it is deemed proper to again present the matter for consideration, with the hope that an agreeable plan might be developed. The subject is one which is best approached with due consideration of the five chief participants, or those principally concerned, namely, (1) The American College of Surgeons, (2) The Hospital, (3) The Attending Physician, (4) The Patient, and (5) The Clinical Pathologist.

The American College of Surgeons is generally regarded as a most powerful constructive agency operating in the field of medicine. Under its influence the chaff in surgery is being winnowed out, unnecessary operations eliminated

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and illegal ones curtailed. Hospitals are transformed from mere boarding houses, to institutions of science. Uniformity in the recording of cases and the bringing together of staff groups at regular intervals for the interchange of views and the scientific discussion of cases, has been accomplished. As most pertinent to this presentation, however, may be mentioned "The Minimum Standard" laid down by the college, which reads: "That diagnostic and therapeutic facilities under competent supervision be available for the study, diagnosis, and treatment of patients, these to include at least a clinical laboratory providing chemical, bacteriological, serological and pathological services." Thus, the seed of scientific medicine is likewise being implanted, and valuable methods of diagnosis, which come within the realm of clinical pathology are now advocated as routine measures, to be applied to all patients who enter the hospitals.

"Competent supervision" is interpreted by the College as meaning a clinical pathologist; and the laboratory procedures which are suggested as a minimum routine for all patients are, qualitative analysis of the urine, cytologic analysis of the blood, and the histologic examination of all tissues, apparently diseased, removed at operation. This, in the opinion of E. W. Willetts of Pittsburg, "is an irreducible minimum," and, adds R. Ottenberg of New York City, "is not too much to demand of a standard hospital," since, to quote from Kano Ikedo of Minneapolis, "The first and primary function of the hospital laboratory is to aid in correct diagnosis and to guide in proper treatment by furnishing to the attending physician certain negative and positive findings"; while H. C. Sweaney of Chicago, voicing the consensus of opinion, believes "that these requirements should be broadened," for, continues the doctor, "the benefits to be derived are too well known, especially when a thoroughly trained man takes the work in hand."

The impossibility for all standardized hospitals to obtain a full-time clinical pathologist may be gleaned from the fact that, according to estimates, there are less than eight hundred clinical pathologists available at the present time; while in the United States there are 806 hospitals with a bed capacity of 100 and over, to say nothing of the numerous smaller ones. Futhermore, many clinical pathologists are occupied with their private laboratories and are hence reluctant to accept full-time positions, especially in view of the uncertain economic state of hospital service. However, Frank W. Hartman of Detroit, is impressed, "that competent supervision would not necessarily mean that the clinical pathologist must be a full-time member of the Hospital Staff," for, says A. H. Sanford of the Mayo Clinic: "His time might be divided with a minimum of two hours a day to be spent in each laboratory" and, adds T. H. Boughton of Akron, Ohio: "A small hospital can usually arrange for such service by cooperation with a larger one, if the expense is too great to maintain a completely equipped laboratory and staff. We have this arrangement with three such hospitals, one in Akron and two outside." Voicing the same sentiment which, in view of the scarcity of workers in this field, is the consensus of opinion of the clinical pathologists, F. C. Narr of Kansas City states

that "For smaller hospitals, I can see no objection to having one pathologist supervising the work of two or three."

As the question in hand pertains to the financing of this new department, our attention will be directed to the manner in which the subject is viewed by the hospitals.

Hospitals generally have adopted the ideals as suggested by the American College of Surgeons. One cannot view the efforts of these institutions to comply with the standards without feeling a sense of pride and satisfaction. By their efforts the standard of medicine is being elevated, in spite of the fact that they have encountered stubborn opposition.

These institutions have had to meet the complaints of patients against the extra charge for routine laboratory work. This has been largely due to the apathetic attitude of some physicians and surgeons toward standardization. But, says V. L. Andrews of Pittsburg: "The public generally is becoming educated to the fact that a laboratory is a great help to the doctor and many of them are demanding laboratory work and are going to doctors, by preference, who have this work done." And, observes Frederick E. Sondern of New York City: "Patients complain against charges for laboratory work only when the attending physician neglects to inform them in advance that this is a necessary expense, just as his fee is a necessary expense." Regarding the fear that has been expressed in some quarters that insistence upon this added expense is resulting in the opening of small nonstandardized hospitals to the detriment of the larger ones which are trying to maintain the standards; Harry J. Corper of Denver, in agreement with the general opinion, says: "There is just as much justification in the hospital's opposition to doing good clinical pathology on the basis of the patients complaining against the extra charge for laboratory work, as there would be on the basis of the patients complaining against the surgeon's or internist's fee—true, some patients complain about both, and poorer grade hospitals will crop up now and then in violation of standards, but we notice that the Mayo Clinic has not become extinct or closed its doors for lack of funds. When the public becomes educated to the fact that first-class service can only be obtained in Class A hospitals with good laboratories, then this cry will cease." While A. H. Sanford of the Mayo Clinic assures us that, "The public are appreciating more and more the value of laboratory work, and will soon ask to go to hospitals where they are not only charged for such service, but where they know they can get good laboratory work. I personally have seen a great change in the attitude of the public toward the Wassermann test alone. A few years ago. I would conceal from the patient the true nature of the test. Now it is very common for a patient to say, 'Are you going to do a Wassermann test?' They are always satisfied when the reply is in the affirmative."

Thus hospitals may be reasonably assured that sentiment is crystallizing on all sides and it is only necessary to maintain a firm stand. All opposition will soon fade into thin air.

Patients have complained with reference to routine laboratory work, that it is unfair to charge for something which they have not requested. They look

to their attending physician for all service essential to their case. In many instances, those in attendance do not advise them with reference to the charge and in some cases have even told them not to pay it. "But," says M. L. Holm of Lansing Michigan, "all patients prefer to go to the better equipped hospitals. We know that patients are in the habit of paying for treatment only, but it is comparatively easy to train them to realize that medical service is not always measured by pills and stitches." As L. S. Lippencott of Vicksburg views it: "Education is the solution, and education must be applied to physicians as well as patients. The physician must be made to realize that laboratory service is necessary and must explain it to his patient. One factor that makes the physician slow in this regard is his unwillingness to admit to his patient that there is something which he cannot do. "Moreover," says F. C. Narr of Kansas City, "every hospital has a right, for the protection of its reputation, to require that every patient admitted to it be adequately studied, in order that a correct and complete diagnosis be made and proper treatment instituted. If the attending physician will not see to this, then the hospital, in self-protection, should." "The physician," thinks Robert F. Maul of Denver, "must be gradually taught that the laboratory is just as essential in the hospital as is the operating room."

Physicians, many of them, are open to censure for their apathy, not to say hostility, toward standardization; especially in the earlier days of the movement. Even fellows of the American College of Surgeons are not without fault in this direction. In the beginning there was almost a solid wall of opposition, characterized not only by indifference but many took the position that there was no precedent to justify the introduction of routine laboratory tests on hospital patients; that if this must be done, a technician might be employed on a small salary in order to obviate the added expense.

"It is usually the man of the old school who takes such a stand," says C. L. Klenk of St. Louis, "the more modern man, if he makes such a statement does so only because he is ignorant of the responsibility of the pathologist." "And," adds Phillip Hillkowitz, of Denver, "technicians cannot interpret the findings any more than we should expect nurses to perform operations." We are reminded by Sanford of the Mayo Clinic, that, "It takes about ten years for the profession to accept a new idea." "There is always opposition to anything that is out of the old order," states W. F. Thomson of Beaumont, Texas; while Ruth Gilbert of the New York Department of Health, at Albany, has to say: "If one compares the present attitude of physicians in regard to laboratory work with their attitude ten years ago, it will be noted that a marked change in favor of clinical laboratory service has occurred, and this tendency will undoubtedly continue, since medical schools are emphasizing more and more the importance of laboratory work. The unfortunate demand in certain localities for 'cheap' laboratory service seems one of the greatest hindrances to progress. Unless the character of the work is satisfactory, it is obviously better not undertaken. Nothing can be worse than false or misleading reports. The fact that an unsupervised technician does certain tests well, may lead physicians to place confidence in work less efficiently done; and thus

tend to discredit laboratory work in general in the minds of the physicians concerned.''

My personal opinion in this matter may be expressed as follows: The technician occupies the same relative position to the clinical pathologist that the nurse does to the surgeon. One would not expect the surgeon to cut sponges, clean instruments, prepare the operating room, make the patient ready for the operation, and clean up after it is over. Technicians, likewise, absorb much of the detail work in connection with the clinical laboratory. Many of them do become proficient in the technic of some of the lesser procedures, but in no case, are they qualified to correlate findings with the clinical condition of a patient, any more than a nurse in the operating room would be qualified to perform a laparotomy. True, many of them might conduct the mechanics of an operation very credibly but it would be unsafe, in the absence of the background of a medical education to permit them to assume the responsibility.

Our first thought, as we approach the question of financing clinical pathology in the modern hospital from the viewpoint of

The Clinical Pathologist, is the avoidance of any measure, the adoption of which might impede the development of clinical pathology as a specialty in medicine. It is to be noted that this specialty has evolved, not in connection with the hospitals, but in private laboratories in the medical communities. The development of the emolument of the clinical pathologist has been through the laboratory fee, which was commensurate with the amount of work involved and the refinement of the clinical diagnostic procedure. Pioneers in this branch have not been fettered by the heavy hand of servitude, but have, in common with their colleagues in other specialties, enjoyed the stimulus of adequate compensation for each transaction. These considerations, therefore, should guide those concerned with the growth of this phase of hospital standardization that due care be exercised to preserve, within the hospital, the same economic status for the clinical pathologist that prevails without.

As there exist various relatively common fees for different surgical procedures, which through custom, have become more or less stable; so likewise, has the force of precedent established a reasonable emolument for many of the operations of the clinical pathologist—as was shown by a recent survey among the pathologists of the country—which definitely established the value which time has placed upon the usefulness of the clinical pathologist. The question has been propounded on several occasions of late as to the amount of compensation which an experienced clinical pathologist should receive for full-time service.

Obviously it would be incompatible with good judgment to expect a surgeon of standing to relinquish his private practice and identify himself exclusively with one hospital at an income of, for example, less than ten thousand dollars per year. And there would be relatively few who would consider such a stipend equal to the sacrifice. As a matter of fact few well grounded surgeons would think of accepting a salaried position of this character. Not only because of inadequate reward, but of its servile limitations as well. To say nothing of the

inevitable precedent of paving the way to state medicine; few of us wishing to become institutionalized.

The clinical pathologist likewise, desiring to maintain his status as a consultant in medicine, will best cooperate with the hospitals in the establishment of adequate laboratory service, on a basis similar to that which obtains with relation to the surgical service or that of internal medicine.

The greatest difficulty in the financing of pathologic laboratories in connection with hospital standardization seems to have proceeded from the confusion arising from the two positions which clinical pathologists, as directors of hospital laboratories, have occupied. Primarily the clinical pathologist, especially in his private pursuits, is a consultant; comparable to other departments of medicine. In this capacity, his position is quite distinct from the director of a laboratory maintained by a hospital for the maintenance of its standard, and its service made mandatory upon the physicians and surgeons enjoying its privileges. The financing of these two positions must necessarily develop in dissimilar ways. It is unnecessary, therefore, to refer to the methods or fees to be applied in consultation practice. The hospital will be compelled to sustain its laboratory and operatives as other additions and improvements are provided for. It would seem most reasonable as a basis for determination that the entire expense of the laboratory, the value of its space, the apparatus and material required, adequate compensation for director and technician, should be determined. This amount should be added to the gross income of the hospital and prorated in addition to the standard charge of accommodations which have prevailed. This implies the recognition of a dual capacity for the clinical pathologist who serves as a director or pathologist in the hospital laboratory. His compensation from the hospital is for his service as an indispensable part of modern hospital standard and is, therefore, essentially different from his employment as a consultant of choice of the attending physician or surgeon of the patient. In the latter instance he is obviously the choice of the physician and patient, while in the former he is the necessary adjunct of the hospital for the maintenance of its standard, and ofttimes an uninvited if not objectionable necessity to the attending physician; hence, the basis for criticism of implied injustice.

This has an analogue in the position occupied by the railroad surgeon or the physician retained by any other industry, for the maintenance of certain standards or ideals. Such men are secured at a stated retainer and their positions as consultants or private practitioners are in no way curtailed. The upward trend of humanitarian ideals requires the industries to establish certain standards of health protection for the promotion of which physicians or surgeons may be retained. Likewise, the advance of scientific medicine has made it necessary for the modern hospital to establish a department of clinical pathology which demands the services of a clinical pathologist.

When, however, the fulfillment of the minimum requirements of standardization have been reached, his position assumes that of a consultant and his remuneration should be determined accordingly.

Referring again to the minimum requirements of the College of Surgeons

which reads "That a clinical laboratory be *available* for the study of cases, etc.," "That a clinical pathologist be in charge and that certain tests be done upon the patients when they enter the hospital." It is apparent that the College does not require that all of the refinements of clinical pathology be furnished to the patrons of the hospital on the same basis as light and heat; but, that they be available; convenient; at hand; so that they may be utilized when required.

In conclusion it seems advisable to emphasize the dual position which has been occupied by the clinical pathologist associated with hospital service.

That the subject of financial support of clinical pathological pursuits essential to modern hospital standardization cannot be satisfactorily determined, without provision for each or both positions in which the clinical pathologist may be employed.

That portion of the service performed as a part of the hospital routine is an obligation of the hospital. Special pathological determinations which are deemed sufficiently necessary by the attending physician and surgeon in individual cases as to be required for the elucidation of the clinical state, or to determine the character and amount of treatment, is an obligation of the patient similar to an opinion rendered upon solicitation of the attending physician and compensation determined accordingly.

When such services assume the character of consultation contributing to the understanding and interpretation of the disease, the fee should be anticipated according to the standards established in private laboratories.

The security of the fee for such special service devolves upon the attending physician, or hospital, or both.

The freedom of consultation within the hospitals should not be restricted; but should be extended to all clinical pathologists of standing according to the preference of those concerned, as obtains in other specialties.

A MODIFICATION OF THE KOLMER REACTION*

By F. O. Huntsinger, Pharmacist, U. S. Navy, Great Lakes, Ill.

THE Kolmer technic in the complement-fixation test has been the subject of extensive research in the laboratory at the U. S. Naval Hospital, Great Lakes, Illinois, for the past eight months, and conclusions arrived at have pointed most favorably to a method which we now employ.

It would seem that in previous articles, appearing in different journals, that the great tendency is to cross check one test against another with the same sera; and meet with apparent failure, in noting an absence of hemolysis (or precipitation) in one test, and complete hemolysis in another, when the reverse should occur.

Many factors can account for this, such as erroneous technic and the presence of unknown bodies, passive or active in origin, which will or will not react

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favorably to all tests. We believe the elimination of these differences without destruction of the luetic body is accomplished in the modified Kolmer reaction test used here.

In a series of tests in which the Noguchi and Kolmer technics were used simultaneously, we made a number of checks on cases which were positive for lues, malaria, tuberculosis (active), tonsillitis, Vincent's angina, and an undetermined febrile case. All were negative, except the luetic cases. When a large amount of serum is employed, inhibition of hemolysis will occur. In using a large amount of serum there is a chance that the fatty content, after the application of heat in the water-bath (56° C.), may have some bearing on the inhibition of hemolysis that occurs. For that reason small amounts of the serum are used unless a five tube test is used (as we frequently use when varying amounts of serum and antigen are employed). A negative serum has never given a positive reaction where the technic was correct.

The elimination of isolysins, or the rare autolysins, can be partially or completely accomplished by the washings of the unheated serum with the washed cells which are to be used in the test.

In the case of the Kolmer the patient's serum can be treated with washed sheep cells and thereby bind the native amboceptor that may be present. In the Noguchi, pooled washed human cells can be employed, exerting their action upon the agglutinins, precipitins and amboceptors, which may be present in the serum of patients who have received blood transfusions, et cetera, and have produced hemolysins (?) therein. The serum thus treated, having such bodies present which have any influence upon the cells to be employed, in the presence of human complement, will produce a haptophore group, provided the complement has not been destroyed. The native hemolysins being bound our test becomes more accurate.

A heterolysin being employed in the test in a known unit against a specific volume of a blood cell suspension, meets with a reduced number of obstacles.

After treating the serum thus, it is inactivated in the water-bath (56° C.) for twenty minutes. In our test, using only two tubes, we employ 0.2 c.c. of human serum in each tube of the Kolmer set up, and only 0.1 c.c. in the Noguchi. In the front tubes we employ 0.1 c.c. of acetone insoluble antigen (unit 0.1 c.c.).

The guinea pig complement is made up in the dilution of one part guinea pig serum to nineteen parts of sterile physiologic salt solution. The unit is determined by titration, with double the amboceptor unit and one mil of the 2 per cent suspension of sheep cells in sterile physiologic salt solution. The complement unit is doubled in the test. Each tube in the test then receives this doubled unit of complement. Shake well and incubate for one hour at 37.5° C. At the end of the hour, we remove the rack containing the tests from the incubator and immediately add the antisheep amboceptor (unit doubled) and 1 c.c. of a 2 per cent suspension of sheep cells. Shake well and incubate for one hour at 37.5° C., shaking the tubes well every fifteen minutes for the first forty-five minutes.

Control tubes consist of the positive 4-plus serum, positive 4-plus spinal fluid, negative serum, negative spinal fluid, antigen control on each antigen

employed, amboceptor control, guinea pig complement control and sheep cell control.

The spinal fluids are not inactivated; five tubes are run with a control on each. Amounts of spinal fluid employed in the tubes are as follows: 0.2 c.c., 0.4 c.c., 0.6 c.c., 0.8 c.c., and 1.0 c.c.

Titrations for the unit amounts to be used in the Noguchi test are the same forms of procedure, except that we employ one-half c.c. of a 2 per cent suspension of human cells. Incubation is for only one-half hour. We then add the antihuman amboceptor (double unit) and one-half c.c. of a 2 per cent suspension of human cells. Shake well and incubate for one-half hour at 37.5° C.

We have used both the Noguchi and the Kolmer tests as checks against one another for a long period of time, but lately have run only the Kolmer.

I wish to thank Commander O. J. Mink (MC), U. S. Navy, for his reading and correcting the original manuscript.

CONCLUSION

1. That the Kolmer is the most reliable of all the complement-fixation tests in the detection of lues.
2. That native hemolysins can be destroyed or grouped by proper treating of the fresh blood serum.
3. That malaria in the Kolmer reaction will not give a positive four-plus reaction.
4. That febrile conditions other than lues will not give a positive Kolmer (Yaws influences this test in the tropics).
5. And that the incubator (37.5° C.) or the water-bath method is preferred to the ice-box fixation method.

REPORT OF AN UNUSUAL CASE OF CONGENITAL CARDIAC DEFECT (COR TRILOCULARE) ASSOCIATED WITH TRANSPOSITION OF PULMONARY ARTERY AND ARCH OF AORTA*

By M. G. Wohl, M.D., Omaha, Nebraska

CASES of cardiac congenital defects in man have been the subject of classic discussion by writers such as: Rokitansky,[1] Lebert-Schroter,[2] Keith,[3] Abbott,[4] Schwalbe.[5] For detailed critical analysis of such cases the reader is referred to the authors mentioned.

The case to be described below is quite unique because of the multiplicity of the cardiac defects, accompanied by transposition of pulmonary artery and thoracic aorta.

Clinical Notes.—Female, white, seven months old, of American parentage in whom no history of syphilis, tuberculosis, or any other chronic disease was elicited. The child was full term and normal delivery. Weight at birth six and one-half pounds. She breathed

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almost immediately on delivery, yet she was noticed to be blue at the time of delivery. The cyanosis kept on increasing with the age of the child and remained until her death.

Past History.—Baby has been blue since birth; her cyanosis getting worse when she cried; she did not thrive. Dr. Newell Jones, Omaha, was called in to regulate her feedings. At the time the doctor saw her, she was three and one-half months old; she was cyanotic, poorly nourished, and had slight clubbing of the fingers. There was a palpable thrill over the precordium and systolic murmur was heard over lower sternum which later became audible all over precordium. Pulse rate varied from 130 to 170 per minute. Her temperature was elevated a degree and a half; a few days prior to her death, her temperature became subnormal.

Autopsy Findings.—The body is that of a female, white infant, seven months old, 58 centimeters in length. There is slight edema of legs and feet. Pronounced cyanosis of lips, face, hands and feet. Marked clubbing of fingers. Thymus normal size. The pleural cavity contains light amber-colored fluid. Lungs moderately congested. No other pathologic findings noticed.

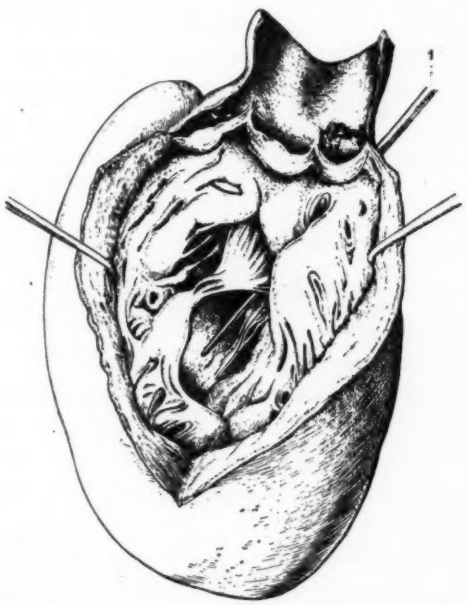

Fig. 1.—Interior of right ventricle. Note thickness of right ventricular wall. Aorta bears three semilunar cusps. *1*, A probe passes through tricuspid orifice from the auricle.

Abdomen: Contains free serous fluid. Normal spleen absent. In the site normally occupied by that organ, there are four accessory splenules; one the size of an olive, the other three the size of a small pea. This is unusual; splenules accessory to normal spleen being not uncommon, however, accessory splenules without the spleen proper is rather rare.

Liver: Weight 190 grams, moderately congested.

Gastrointestinal tract, apparently normal. Gall bladder and pancreas normal; kidneys show cloudy degeneration. Adrenals apparently normal. Sex organs normal. Abdominal aorta normal.

Heart: Pericardium free from adhesions; contains 15 c.c. of clear yellowish fluid. The heart itself is enlarged, weighing 82 grams, and measuring diagonally 9 by 7½ cm. The enlargement is due to hypertrophy of right ventricle and dilated auricles.

The most striking anomaly seen upon the anterior aspect of the heart is the origin of the aorta, which arises from the right ventricle, at the usual site of origin of the pulmonary artery.

The pulmonary artery arises from the left ventricle at the normal place of origin of the aorta. It does not communicate with the ventricular cavity proper, as it is atresic in its lowest 2 cm.; its lumen ending blindly at site of atresia. The inferior vena cava enters the auricle from below at normal site. The superior vena cava, however, opens abnormally wholly into left ventricle. The aorta is 1.5 cm. in diameter at its base and gives off two main coronary branches. The left coronary is seen anterior to the pulmonary artery, passing posteriorly to auricles where it divides into an auricular and anterior circumflex branch. The ductus arteriosus is patent throughout, measuring 1 by 0.5 cm. and connects pulmonary artery with aorta, just proximal to primary division of the latter.

Fig. 1 shows interior of right ventricle. The right ventricle is hypertrophied and dilated; its wall being 2 cm. thick and forming the whole cardiac apex. The aorta, bearing three semilunar cusps of equal size but

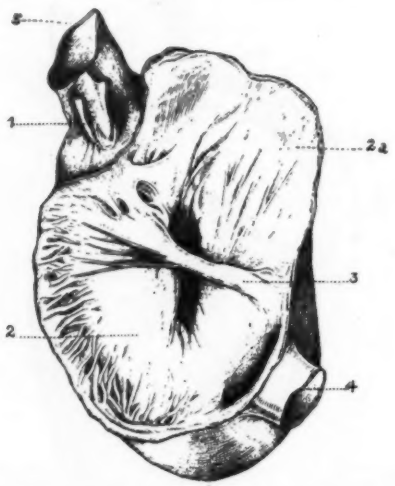

Fig. 2.—Interior of auricular portion of heart. A coronal section has been removed, exposing the interior of both auricles from above.
1, Atresic pulmonary artery; *2*, *2-a*, interior of auricles bisected by muscular band; *3*, defective auricular septum; *4*, inferior vena cava, opening into right auricle from below; *5*, superior vena cava, opening into left auricle.

with thickened contracted borders, rises from the conus of the right ventricle in the position normally occupied by the pulmonary artery, as noted above. The conus is short and its muscular pillars unusually irregular and hypertrophied. A large tricuspid valve having well formed infundibular septae is seen. Its chordae tendinae are fused and thickened. The tricuspid orifice is dilated, giving rise to an insufficiency. The left ventricular cavity is markedly diminished as compared with the right, its wall is also thin, measuring 0.2 cm. The pulmonary artery, already described as arising from normal atresic site, has a thin wall, and is of diminutive size ending below in a glistening culdesac.

The common auricular cavity is partially divided by a narrow thickened muscular band that traverses it obliquely above the center, anteroposteriorly at about normal lines of attachment. This band is strongly attached by its extremities to the musculature of the auricles; it sends on the right side several additional chords, tendinous in nature, to be inserted in a fanshaped fashion on

posterior auricular wall. Aside from this band there is no other septal tissue. The auricles communicate widely, therefore, with each other above and below the band. The band represents apparently an incomplete septum primum. The inferior vena cava enters the right auricle from behind and below to the right of the defective septum. The two auricular lumina, therefore, form a common cavity in wide communication above and below this band. The interventricular septum is complete, separating the right ventricle wholly from the left. The left ventricle which is not shown in the drawing presents walls strikingly thin and weak in comparison with the right. The heart has, therefore, two separate ventricular cavities each opening into a common auricular lumen.*

ANATOMIC DIAGNOSIS

Complete reversal of origin of aorta and of pulmonary artery that arise from normal sites of each other. Aortic insufficiency resulting from the fact that the small contracted aortic valves do not meet, as described above; aplasia and diminution of left ventricle with atresia of mitral orifice; hypertrophy and dilatation of right ventricle. Auricular septum markedly defective above and below. Apparently persistent and much enlarged ostium primum and secundum. Tricuspid orifice dilated. Accessory splenules. Passive congestion of liver. Cloudy degeneration of kidneys.

COMMENT

We are dealing with a rare cardiac anomaly. There are only ten cases of defects of upper part of septum described in the literature.[6]

From a critical survey of the cases reported, it would seem that defects in the interauricular septum are commonly associated with hypoplasia of the aorta and dilatation of the pulmonary artery. Our case is unique in that the aorta and pulmonary artery, were transposed; the aorta was dilated and pulmonary artery stenosed. The pulmonary stenosis would have caused immediate death of the child were it not for the presence of the open ductus arteriosus. This, doubtless, permitted a retrograde flow of blood from aortic arch to pulmonary arterial system which was normal above the site of atresia already noted; the blood, therefore, reached the pulmonary arterial system in an unusual manner. The mechanism in production of this auricular defect may be best understood through the application of the principles of the normal embryologic development as first pointed out by Rokitansky.[7]

"The auricular septum is known to develop in two parallel planes (Born, His); which grows downward from the roof of the common auricle at a very early stage. Of these two, that developing first, the septum primum, lies on the left side; as it grows downward, an opening remains for some time at its lower border above the portion of the interventricular septum formed by the endocardiac cushions. This is known as the ostium primum. Gradually, as the primary septum grows downward, this ostium primum closes in, and a second opening appears above, in the upper part of the primary septum. This is the ostium secundum or foramen ovale which is guarded by the septum secundum.

*It is to be regretted that during preparation of the drawings the specimen became mislaid and other detailed study of it became impossible.

The latter arising from the right auricle to the right of the septum primum, arches downward, acting as a valve in the embryo and forming annulus ovalis of postnatal life."

The connection of the right and left auricular lumina below and above the auricular band described above is apparently a partially persistent ostium primum. The opening below the band is due to failure of this septum to grow down and fuse with the septum below. This assumption is based upon the fact that normally the closing of the ostium primum, by a downward growth of the septum primum, effects a permanent separation of the auricles at this site. The absence of auricular septum above this band is probably the result of an abnormally large ostium secundum or foramen ovale, in the formation of which, the whole upper part of the septum as well apparently as the septum secundum have disappeared. The transposition of the vessels can be accounted for by deviation of aortic septum within the truncus aorticus and by faulty union with the ventricular cavities. That pulmonary stenosis is due to congenital defect rather than to intrauterine endocarditis seems probable, on account of the presence of the other cardiac defects.

I wish to express my appreciation to Dr. Maude E. Abbott and Dr. James McDonald for valuable suggestions made during the preparation of the paper.

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PRODUCTION OF IMMUNITY WITH A SOLUBLE ANTIGEN OF THE PNEUMOCOCCUS*

By N. S. Ferry, Ph.B., M.D., and L. W. Fisher, B.S., Detroit, Mich.

During the past two years the writers have been carrying on some extensive experimental work for the purpose of determining the immunizing properties of bacterial antigens prepared after various methods; and as a result of this work have reported some unusual and unexpected findings.[1]

Having tested over three hundred antigenic preparations from several different organisms during this time, we have been compelled to modify in many respects our previous conceptions of bacterial antigens, especially as to their origin.

While testing broth cultures of some of the so-called endotoxin producing organisms, as the typhoid bacillus, colon bacillus, pneumococcus and others, it was found that the antigenic properties of the filtrates were of extremely high value, providing they were obtained from growths incubated at the right temperature and for the proper length of time. The optimum temperature proved to be about 37° C. while the incubation time, which gave the best results, was between eighteen and twenty-four hours.

In an endeavor to carry out these experiments as qualitatively as possible, twenty-four broth cultures were divided into three parts or fractions; one to be tested as such, one to be centrifugalized for the preparation of the filtrate fraction, termed the "centrifugate" and one to be centrifugalized for the sediment (diluted to original volume with salt solution). These three fractions were sterilized by the addition of 0.3 per cent cresols. Their antigenic properties were determined by injecting them intravenously into rabbits in three or four doses and testing the serums, according to various serologic methods, for antibody content. The results showed that the filtrates in many instances equalled and, in some cases, were superior in antigenic value to both the original broth culture and the sedimented bacteria. In practically all tests the sedimented bacteria were found to be inferior in antigenic properties to the whole culture and in many instances also to the filtrate. (See Table I.)

It was found that if broth cultures were incubated over twenty-four hours, the antigenic properties diminished proportionately, showing that there must be something formed in the media or secreted by the organisms antagonistic to the antigens. (See Table II.)

These results have demonstrated quite conclusively that the antigens of these organisms are found in the filtrates of broth cultures in a high degree of availability and have also convinced us that the antigens are not entirely endotoxic in nature. They must be more or less loosely bound to the cells.

*From the Medical Research Laboratories, Parke, Davis & Co., Detroit.
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From these findings, it was concluded that if the antigens were loosely bound to the cells or were a component of the ectoplasm, they would be more apt to be found in greater concentration in an aqueous solution obtained by merely washing the organisms grown on solid media than if an attempt were made to extract them by chemical or physical means.

In an attempt to prove this point washings were prepared as follows: The organisms were grown on solid media for twenty-four hours, taken off in salt solution, shaken in mechanical shaker for a few minutes and then immediately

TABLE I

TEST OF THE ANTIGENIC VALUE OF BROTH CULTURES OF VARIOUS ORGANISMS CONTAINING 1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA		
METHOD OR PREPARATION	AGGLUTINATION	COMPL.-FIX.	BACTERIOTROPIN
Broth culture of B. typhosus (1000 million organisms per c.c.)	1:18000	1:10000	1:2800
Centrifugate from broth culture	1:16000	1:10000	1:6000
Sediment from broth culture diluted to original volume	1:4000	1:700	1:450
Broth culture of B. coli	1:18000	1:150	
Centrifugate from broth culture	1:12000	1:600	
Sediment from broth culture diluted to original volume	1:900	1:200	
Broth culture of pneumococcus Type I	1:80	0	
Centrifugate from broth culture	1:70	0	
Sediment from broth culture diluted to original volume	0	0	
Broth culture of streptococcus	1:500	1:10	
Centrifugate from broth culture	1:600	1:10	
Agar suspension (1000 million organisms per c.c.)	1:800	1:60	
Agar washings	1:800	1:50	
Broth culture of gonococcus	1:400	1:10	
Centrifugate from broth culture	1:400	1:10	
Agar suspension of equal number of organisms	1:500	1:100	
Agar washings	1:600	1:100	

TABLE II

TEST OF THE INFLUENCE OF THE INCUBATION TIME, PEPTONE AND SALT UPON BROTH CULTURES OF B. COLI

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Plain Broth		
Incubated 5 hours (centrifugate)	1:150	1:10
" 24 hours "	1:8000	1:600
" 5 days "	1:300	1:50
" 10 days "	1:300	1:50
Plain broth without peptone		
Incubated 5 hours (centrifugate)	1:60	1:40
" 24 hours "	1:150	1:40
" 5 days "	1:60	1:10
" 10 days "	1:250	1:10
Plain broth without peptone and salt		
Incubated 5 hours (centrifugate)	1:40	1:15
" 24 hours "	1:40	1:10
" 5 days "	1:200	1:10
" 10 days "	1:80	1:15

passed through a Sharples centrifuge. The entire operation did not consume over half an hour from the time the growth was taken from the incubator to the final stage in the process.

These washings have been tested together with antigens prepared by other well-known methods and have been found to be extremely high in antigenic value. When injected into rabbits, following the method described above for testing broth cultures, the washings were found capable of producing antisera of very high titer. (See Tables III and IV.)

The washings have also been used with success in the rôle of antigens in the complement-fixation test. (See Table V.)

Recently[2] with these same washings prepared from the Streptococcus hemolyticus scarlatinae we have been able in collaboration with Dr. Pryer to produce a positive skin reaction in individuals susceptible to scarlet fever (Dick reaction), which reaction would become negative if the washings were

TABLE III

TEST OF THE ANTIGENIC VALUE OF VARIOUS ANTIGENS PREPARED FROM THE TYPHOID, PARA. A AND PARA. B, BACILLI DILUTED SO THAT ALL CONTAINED 1000 MILLION ORGANISMS PER C.C.

ANTIGENS		TITER OF IMMUNE SERA	
METHOD OF PREPARATION		AGGLUTINATION	COMPLEMENT FIXATION
Typhoid vaccine		1:9000	1:1000
Typhoid-paratyphoid vaccine comb.	B. typhosus	1:6000	1:100
	B. para. A.	1:5000	1:50
	B. para. B.	1:8000	1:250
Typhoid-paratyphoid washings	B. typhosus	1:16000	1:750
	B. para. A.	1:2000	1:750
	B. para. B.	1:20000	1:750
Typhoid-paratyphoid broth centrifugate	B. typhosus	1:10000	1:175
	B. para. A.	1:3000	1:60
	B. para. B.	1:12000	1:750
Typhoid glycerol vaccine	B. typhosus	1:6000	1:90
Typhoid serobacterin	B. typhosus	1:5000	1:75
Detoxicated T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35
Residual T.A.B. vaccine	B. typhosus	1:2000	1:25
	B. para. A.	1:20	1:5
	B. para. B.	1:200	1:35

TABLE IV

TEST OF THE ANTIGENIC VALUE OF VARIOUS GONOCOCCUS ANTIGENS DILUTED SO THAT ALL CONTAINED THE SAME NUMBER OF ORGANISMS

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Agar washings of gonococcus	1:2000	1:100
Broth centrifugate of gonococcus	1:400	1:50
Gonorrhea (comb.) vaccine	1:900	1:90
Residual gonococcus vaccine	1:80	1:10
Gonococcus glycerol vaccine	1:100	1:70
Gonococcus serobacterin	1:30	1:50

TABLE V

TEST OF VARIOUS TYPHOID ANTIGENS WHEN USED AS SUCH IN THE COMPLEMENT-FIXATION TEST AGAINST A KNOWN ANTITYPHOID SERUM

ANTIGENS	IMMUNE SERA
METHOD OF PREPARATION	HIGHEST DILUTION FIXING COMPLEMENT
Mixture of broth centrifugate and agar washings of B. typhosus	1:90
Agar washings of B. typhosus	1:80
Agar washings of typhoid-paratyphoid mixture	1:60
Broth centrifugate of typhoid-paratyphoid mixture	1:90
Broth centrifugate of B. typhosus	1:70
Autolysates of B. typhosus	0

TABLE VI

TEST TO DETERMINE THE INFLUENCE OF REPEATED WASHING UPON THE ANTIGENIC VALUE OF SUSPENSIONS MADE FROM THE WASHED ORGANISMS. B. TYPHOSUS WAS USED AS THE TEST ORGANISM. 1000 MILLION ORGANISMS PER C.C.

ANTIGENS	TITER OF IMMUNE SERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Agar suspension	1:8000	1:325
Agar washing	1:11000	1:500
Washed organisms	1:4000	1:600
Broth growth	1:7000	1:300
Broth centrifugate	1:8000	1:250
Broth sediment	1:35000	1:600
Broth sediment washed	1:3000	1:500
Washings from antigen No. 63	1:1500	1:80

mixed with convalescent scarlet fever serum, similar to the results obtained by the Dicks of Chicago,[3] Zingher of New York,[4] Branch and Edwards,[5] Gatewood[6] and others with the Dick toxin.

Returning to the sedimented bacteria from which the washings were obtained, it was found, as with the sediment from the broth cultures in the first experiment, that the washed organisms were relatively low in antigenic properties. These sedimented bacteria washed the second time have yielded antigens even lower in antigenic properties than the sediment after the first washing and the second washings from this sediment have also suffered more or less in antigenic properties. (See Table VI.)

In carrying this work further, autolysates were prepared from the organisms, both before and after being washed, and it was found that they were even lower in antigenic value than the second washings from the unautolyzed sediment. This was especially marked of the autolysates from the washed organisms, showing that washings did not depend upon the autolyzation of the organisms for their antigenic properties. (See Table VII.)

Another experiment, which proves conclusively the antigenic value of these washings and centrifugates and would also tend to lend some weight to the suggestion that antigens are nonprotein in nature, was carried out by precipitating several different washings and broth centrifugates with various substances until a protein-free reaction was produced. These apparently protein-

TABLE VII

TEST TO DETERMINE THE INFLUENCE OF WASHING ORGANISMS, BOTH WITH AND WITHOUT LONG SHAKING, UPON THE ANTIGENIC VALUE OF THE AUTOLYSATES OF THE SEDIMENTED BACTERIA. SUSPENSION OF B. TYPHOSUS WAS USED, 1000 MILLION BACTERIA PER C.C.

ANTIGENS	TITER OF ANTISERA	
METHOD OF PREPARATION	AGGLUTINATION	COMPLEMENT FIXATION
Agar washings	1:12000	1:750
Broth centrifugate	1:12000	1:375
Autolysates from residue of agar washings	1:3000	1:100
Autolysates from residue of broth centrifugate	1:7000	1:500
Agar washings after shaking suspension 24 hr.	1:5000	1:500
Autolysates from residue of previous antigen	1:2000	1:100
Autolysates from agar growth not previously extracted by shaking	1:7000	1:150

free antigens were injected into rabbits, according to the usual method, and the serums tested for the presence of antibodies with positive results in several cases. (See Table VIII.)

From these experiments, it was suggested that the antigens of the organisms studied were more liable to be ectoplasmic than endoplasmic in origin, and for that reason this type of an antigen has been termed an "ectoantigen."

The more recent work which we wish to report at this time includes experiments carried on with washings or ectoantigens prepared from Type I pneumo-

TABLE VIII

TEST OF THE ANTIGENIC VALUE OF ANTIGENS APPARENTLY PROTEIN FREE

ANTIGENS			TITER OF IMMUNE SERA	
ORGANISMS	METHOD OF PREPARATION	METHOD OF PRECIPITATION	AGGLUTINATION	COMPLEMENT FIXATION
B. typhosus	Washings	Phosphotungstic acid	1:200	1:25
B. typhosus	Broth centrifuge	Uranium acetate	1:80	1:25
B. coli	Washings	Acetone	1:100	1:5
B. coli	Broth centrifuge	Uranium acetate	1:200	1:50
B. coli	Broth centrifuge	Phosphotungstic acid	1:2000	1:75
Pneumococcus	Phenolized centrifugate	Acetone	1:40	1:10
Pneumococcus	Agar washings	Acetone	1:80	1:25
Pneumococcus	Phenolized centrifugate	Phosphotungstic acid	1:40	1:10
Pneumococcus	Phenolized centrifugate	Uranium acetate	1:40	1:10
Streptococcus	Agar washings	Acetone	1:800	1:25
Streptococcus	Phenolized centrifugate	Acetone	1:400	1:5
Gonococcus	Agar washings	Acetone	1:100	1:10

coccus. The organism used for all the experiments was a strain obtained from the Hygienic Laboratory, Washington, for the preparation of Type I antipneumococcic serum. It has repeatedly been tested, and according to all known serologic tests, has proved to be true to type with a minimum fatal dose to white mice of from one hundred millionth to one billionth cubic centimeter of a twenty-four hour broth culture.

In preparing the washings the seed cultures were grown on blood agar and transplanted to large flasks of ascites agar. These were incubated twenty-four hours and washed off with salt solution and divided into five lots.

TABLE IX

TEST TO DETERMINE THE VALUE OF TYPE I PNEUMOCOCCUS WASHINGS BY MEANS OF ACTIVE PROTECTION TESTS ON WHITE MICE

LOT	MOUSE	DILUTION OF CULTURE	RESULTS
I	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
II	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
III	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	Died
	5	0.001	"
	6	0.01	"
	7	0.1	"
	8	1.	"
IV	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	Died
	6	0.01	"
	7	0.1	"
	8	1.	"
V	1	0.0000001	Lived
	2	0.000001	"
	3	0.00001	"
	4	0.0001	"
	5	0.001	"
	6	0.01	Died
	7	0.1	"
	8	1.	"

M. F. D. of culture—0.000000001 c.c.

Lot I. The suspension was subjected to a temperature of 60° C. for one hour, after which it was treated by the usual method for the preparation of washings.

Lot II. This was washed first and then the washings were subjected to a temperature of 50° C. for one hour.

Lot III. This was treated as Lot II but heated only to 40° C. for one hour.

Lot IV. This suspension was treated with 0.3 per cent cresols and allowed to stand twenty-four hours before treatment as a washing.

Lot V. This was treated at once without heat or cresols, according to the previously described method of preparing washings.

To all of the final products except Lot IV, 0.3 per cent cresols were added as a preservative. White mice were given two doses of 0.5 c.c. of these extracts

subcutaneously one week apart and one week after the last dose were injected intraperitoneally with varying doses of the live organisms. (See Table IX.)

Results.—The results showed that Lot I which had been heated to 60° C. for one hour before being washed protected against 10,000 M. F. D. and the same was true of Lots II and III which had been heated to 50° C. and 40° C., respectively. Lot IV which had been sterilized by the addition of 0.3 per cent cresols for twenty-four hours before washing, protected against 100,000 M. F. D. while Lot V extracted a few minutes with salt solution, protected against at least 1,000,000 M. F. D.

Discussion.—From these results it is evident that an aqueous extract of Type I pneumococcus may be obtained from the washings of the organisms, fairly stable at a temperature of 60° C. for one hour with antigenic properties extremely high, without resorting to chemical extraction; and suggests the possibility of the use of such an extract for prophylactic purposes against pneumonia in the human.

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COMPLEMENT FIXATION IN TUBERCULOSIS WITH PARTICULAR REFERENCE TO A GROUP OF 102 POSITIVE REACTIONS*

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DURING the past few years it has been the purpose of this laboratory to study the complement-fixation reaction as applied to the blood sera of patients, in the diagnosis of tuberculous disease. It is recognized that the test has peculiar limitations and the purpose of this study has been to determine the specificity of a positive reaction. The problem has been attacked from the viewpoint of determining the value of the positive reaction as obtained from the blood serum.

With one particular lot of antigen, which after extensive trial, with many tuberculous and clinically nontuberculous sera, was found to possess desirable antigenic properties, the blood sera of 617 patients were subjected to the test. Of the number tested, 102 gave positive reactions of either "+++" or "++++" degree (basis: four plus representing complete inhibition of hemolysis).

Of the more than 400 patients giving a negative reaction to the test, it was recognized that many of the number were tuberculous. However, the purpose

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of the present discussion is merely to determine the incidence of tuberculous disease among those individuals giving a positive reaction.

The technic of the reaction as made in this particular study was as follows:

a. *Antigen.*—Necessarily an essential element in making a satisfactory test is a reliable antigen of established antigenic properties. Several strains of B. tuberculosis were grown in flasks of nutrient glycerin bouillon in large volume, then removed and washed with saline solution, centrifuged and later removed to a drying chamber. Masses of the partially dried bacilli were then transferred to a mortar and ground with a pestle for more than three hours, only slight amounts of distilled water being added. Smears made of the material and stained with fuchsin showed considerable disintegration of many bacillary bodies. After the grinding process, distilled water was added in amount sufficient to make the resulting mixture markedly opaque. One half of one per cent of phenol was added as a preservative and the mixture heated in a water-bath to 70° C. for twenty minutes. For use, one to ten dilutions were made in physiologic saline solution and standardization effected by titration with known positive and negative sera. The dose of antigen for use in the test was considered as an amount two times the least amount giving complete inhibition with a positive serum, in the event that twice that tentative amount did not inhibit hemolysis with a negative serum. Lots of antigen showing excessive anticomplementary or irregular antigenic properties were discarded.

b. *Patient's Serum.*—Clear blood serum was regularly inactivated at 56° C. for thirty minutes on the day following withdrawal.

c. *Complement.*—Pooled blood sera from two or more guinea pigs. The serum from each pig having been previously tested for fixability with this antigenic system. For use, one to ten dilutions were made in physiologic saline solution.

d. *Hemolytic Amboceptor.*—Blood serum of a rabbit highly immunized to sheep erythrocytes.

e. *Sheep Cells.*—Obtained from the sheep and used as a 5 per cent suspension of the washed cells.

The tests were performed in a manner closely paralleling the classical Wassermann test. Two units of complement, the dose of antigen and 0.2 c.c. of patient's serum were incubated for one hour in the water-bath at 37° C., whereupon two units of hemolytic amboceptor and 0.5 c.c. of the cell suspension were added to each tube and readings made after a thirty minute period of secondary incubation. Concerning the length of the primary incubation period, it was noted that a greater degree of specific fixation was obtained with an incubation period of one hour at 37° C. than was obtained at "ice box" temperature (6° C.) for four hours, a distinct variation from the experience frequently encountered in working with bacillary antigens.

An attempt was made to classify the various physical findings on the 102 patients giving a "+++" or "++++" reaction to the test. Competent examiners will frequently disagree on a diagnosis of clinical tuberculosis; hence, the clinical diagnoses as listed cannot be considered as an absolute index to the accuracy of the test. Data considered in basing a diagnosis of clinical tuberculosis were obtained from the history, temperature and weight charts, physical examinations, sputum examinations, Roentgen plates and interpreted in manner as is customary among most authorities on tuberculosis.

Pulmonary tuberculosis, active, with B. tuberculosis present in the sputum.	Cases 55
Pulmonary tuberculosis, active clinically, well defined cases but with negative sputum.	Cases 22
Pulmonary tuberculosis quiescent clinically, well defined evidence of former tuberculous process	Cases 21
Pulmonary tuberculosis negative clinically. Syphilitic. Wassermann positive.	Cases 1
Pulmonary tuberculosis negative clinically. Diagnosis: Laryngitis	Cases 1
Pulmonary tuberculosis negative clinically. Diagnosis: Asthma	Cases 1
Pulmonary tuberculosis negative. Otherwise negative clinically	Cases 1

The results here given, though encouraging, are not of sufficient scope to prognosticate the value of the reaction. In surveying these cases the purpose has been to determine, in so far as is clinically possible, the incidence of definite tuberculous disease among individuals giving a positive reaction to the test.

Concerning the so-called "cross-fixation" or nonspecific fixation with luetic sera, frequently reported, it was noted in this investigation that ten of the individuals gave a positive Wassermann reaction concomitantly; however, in each case diagnoses of dual disease: viz., syphilis and tuberculosis were made in all except one, who was considered as being clinically tuberculosis-free. Of the ten cases referred to, five were available for specific therapy and after intensive antiluetic treatment, with the disappearance of Wassermann reaction, it was noted in all cases that positive reactions with the complement-fixation test for tuberculosis persisted. So it is thought that with the use of a properly prepared antigen and properly controlled technic, nonspecific fixations with luetic sera need not be frequently encountered.

In making the test with the blood sera of patients who were in a far advanced stage of tuberculosis, it was noted that negative reactions were obtained in approximately 50 per cent of such cases. Presuming that we are dealing with a true antibody reaction, this anomaly is readily explained by the supposition that enormous amounts of tubercle antigen, are elaborated into the blood stream, incident to the extensive tuberculous process in a terminal case. By absorption, neutralization or other physiochemical processes the "reaction-bodies" necessary for the fixation of complement are rendered inert, hence, when such a condition is established, negative complement-fixation reactions will follow.

SUMMARY

1. An antigen preparation for the test should be accepted only after thorough preliminary trial demonstrating satisfactory antigenic qualities. The amount to be used in the test can be determined only after careful experimenta-

tion and must be such as permits a very fine quantitative adjustment between complement, antigen and patient's serum.

2. Several control sera should be regularly employed to detect evidence of false fixation, antigen deterioration, etc.

3. Limited observations, herein reported, would tend to promote the belief that positive reactions as obtained with the test when correctly performed, indicate with a high degree of accuracy the presence of tuberculosis "reaction-bodies" irrespective of whether the disease is then clinically active or quiescent.

THE INFLUENCE OF EXPERIMENTAL ATHEROSCLEROSIS UPON THE SYSTOLIC BLOOD PRESSURE IN RABBITS*

By Shepard Shapiro, M.D., and David P. Seecof, M.D., New York City, N. Y.

INCREASES in systolic blood pressure have been reported in rabbits following the prolonged feeding of cholesterol in oil, or of cholesterol containing substances administered for the purpose of producing atherosclerosis, by Fahr,[1] Van Leersum,[2] Schmidtmann[3] and Schönheimer.[4]

Three different methods for measuring the blood pressure were used by these authors. The method employed by Fahr[1] is as follows: A rubber cuff connected to a manometer is placed around the abdomen of the rabbit; air is forced into this cuff until the abdominal aorta is occluded, as indicated by the disappearance of the femoral pulse. The pressure in the cuff is then gradually released, and the instant the first femoral pulse returns the reading is taken and recorded.

In seven of the ten rabbits to which he had fed egg yolk and milk and which showed atherosclerosis at necropsy, Fahr observed increases in the blood pressure. In two of four rabbits which he had suspended, head down, daily, he observed similar elevations in blood pressure. The arteries in these latter four rabbits showed no atherosclerosis. Fahr observed cardiac hypertrophy in those rabbits in which he found hypertension following egg yolk feeding; and claims, further, that there was in these animals a parallelism between the degree of atherosclerosis, the cardiac hypertrophy, and the hypertension.

There is an obvious objection to Fahr's method. Constriction of the rabbit's abdomen is unphysiologic and *per se* gives rise to uncontrollable alterations in blood pressure.

Van Leersum[5] used a method which he published in 1911, and which briefly is as follows:

The carotid artery is isolated and a rubber tube passed around it. The wound is then permitted to heal with the rubber tube in position. At the time the reading is taken (from two to four weeks after the operation) a rubber cuff is joined to the tube and held in place by forceps. This cuff is connected to a

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rubber bulb and a manometer, and air is forced into the tube until the artery is occluded. The pressure is then gradually released and at the instant the first pulsation is felt by palpation of the vessel the reading is made. This is recorded as systolic pressure. By this means Van Leersum[2] observed an increase in systolic blood pressure of 30 per cent after feeding powdered liver to rabbits for long periods of time.

This method offers a very direct means for determining blood pressure and would be an excellent one, provided the artery could be thus isolated without altering the compressibility of its walls through inflammatory reactions. This objection seems to render the method both impractical and prohibitive.

Van Eweyk and Schmidtmann,[6] therefore, in 1922, published the following method:

The rabbit is fastened to a board. The circulation in one of the forelegs is occluded by means of a tourniquet. A rubber cuff, which is connected to a bulb and manometer, is then placed around the upper portion of the leg. Air is forced into the cuff until the manometer reads 160 mm. mercury. The tourniquet is then removed. A small incision through the skin is made below the point of occlusion and the pressure in the cuff is gradually reduced. When the blood appears in the small arteries of the exposed fascia, the manometer is read and this reading recorded. The incision is sewed up and iodine applied. Using this method, Schmidtmann[3] observed a rise in blood pressure following the feeding of powdered liver in six rabbits, confirming in this one respect Van Leersum. He reports, further, that the blood pressure fell after powdered liver was discontinued and rose again when feeding with liver was resumed.

At necropsy, Schmidtmann found intimal changes present in the aortas of these rabbits. It should be emphasized that Van Leersum, in the eight rabbits in which he observed elevations in blood pressure (as determined by his method) following feeding with powdered liver, failed in all cases to find atherosclerosis on postmortem examination. Schmidtmann also claims that there is a direct relationship between the severity of the vascular lesion and the height of the blood pressure; and concludes that the cholesterol contained in the powdered liver is the pressor substance because he found, (a) administration of dried muscle to three rabbits to have no noteworthy effect and (b) cholesterol in oil fed to two rabbits (1 gram twice a week for nine and twelve weeks respectively) to cause an increase in blood pressure of about 50 per cent.

Using Van Eweyk and Schmidtmann's[6] method Schönheimer[4] also recently reported an increase in blood pressure of about 32 mm. mercury (from normal of 80 to 112) following administration of cholesterol in oil to several rabbits.

The method of Van Eweyk and Schmidtmann is, we believe, open to the following criticisms:

1. The rabbit must be fastened to a board. This entails a certain amount of struggling which may increase the blood pressure.

2. An incision through the skin alters the blood flow to the part which, in turn, influences the blood pressure as determined in that portion of the limb at that time.

3. It would be difficult, simultaneously, to record the manometer reading and observe the first appearance of blood in the incised area.

If by a more reliable method, free from the objections offered above, the findings of Fahr, Schmidtmann and the others could be confirmed, some additional light might be shed on the age-old controversy of the relationship between hypertension and the development of atherosclerosis.

In 1923, Anderson[7] described a simple apparatus for determining the systolic blood pressure in the central artery of the rabbit's ear. It occurred to us that by this means we could study the blood pressures of rabbits over long periods of time. Using Anderson's method, we have studied the systolic blood pressures of seven rabbits which were being fed lanolin for the production of

TABLE I

RABBIT NO. SEX	AGE AT DEATH (DAYS)	PREVIOUS OPERATIVE PROCEDURES	PERIOD LANOLIN FEEDINGS (DAYS)	NO. OF READINGS	SYSTOLIC BLOOD PRESSURE			DEGREE ATHEROSCLEROSIS	REMARKS
					HIGHEST	LOWEST	AVERAGE		
386 F	828*	Rt. & Lt. Suprarenalectomy	110	6	100	80	87	Slight	Readings 3 mo. after operations
408 M	664	Thyroidectomy Rt. & Lt. Suprarenalectomy	268	5	95	81	86	Marked	Readings before and 50 days after operations
416 F	573	Splenectomy	173	5	105	85	92	Marked	Readings 6 mo. after operations
417 F	603	Rt. & Lt. Suprarenalectomy	272	5	105	80	91	Marked	Readings before and 50 days after operations
423 M	482	Thyroidectomy	183	2	85	80	83	Moderate	Readings 3 mo. after operations
451 F	591	Splenectomy Rt. & Lt. Suprarenalectomy	100	5	100	80	87	Slight	Readings 4 mo. after operations
454 M	533	Splenectomy Rt. & Lt. Suprarenalectomy	106	6	100	88	90	Slight	Readings 4 mo. after operations
377 F	850*	Rt. & Lt. Suprarenalectomy	100	3	97	87	92	None	Readings 6 mo. after operations
490 M	512	Rt. & Lt. Suprarenalectomy	100	3	105	90	96	None	Readings 6 mo. after operations
441 F	631	Thyroidectomy Rt. & Lt. Suprarenalectomy	50	5	100	87	90	None	Readings 3 mo. after operations
463 M	419	Rt. & Lt. Suprarenalectomy	60	5	105	82	92	None	Readings 3 mo. after operations
411 F	603	Rt. & Lt. Suprarenalectomy	10	4	102	82	92	None	Readings before operations
414 F	647	Rt. & Lt. Suprarenalectomy	10	3	95	80	88	None	Readings before operations
374 M	629	Rt. & Lt. Suprarenalectomy	45	6	98	65	77	None	Readings 3 mo. after operations
497 M		Rt. & Lt. Suprarenalectomy	None	4	105	95	98	None	Readings before operations
432 F		None	None	3	98	87	93	None	
499 M	145	None	None	3	85	75	80	None	
501 M	535	Snip Thyroid removed	None	4	91	85	88	None	

*Approximate.

atherosclerosis, and of a number of control rabbits kept under similar hygienic and dietary conditions. Among these controls were rabbits which were subjected to the same operative procedures as the above seven, and some which were fed lanolin for varying periods of time from 10 to 100 days, but which at autopsy showed no atherosclerosis.

The blood pressure readings were made at irregular intervals during the course of the experiments. In order to avoid personal bias, the readings were made and recorded before the number of the rabbit was identified. The results of these blood pressure readings are summarized in Table I.

DISCUSSION

The average systolic blood pressure in the central artery of the rabbit's ear, according to Anderson,[7] varies between 75 and 90 mm. mercury. Our findings in rabbits, which we used as controls, agree with those of this investigator.

In each of the seven rabbits with atherosclerosis the average of all the readings fell within normal limits. The average blood pressure of these rabbits was 88, while in the 11 control rabbits it was 89.

It is true that the animals with marked atherosclerosis occasionally gave slightly higher readings than the controls. We cannot, at present ascribe any importance to this, although the possibility must be pointed out that experiments carried out over longer periods of time than ours, might show more definite rises. The point we wish to emphasize is, that although there was no significant hypertension at the end of the experimental periods, the vascular lesions at autopsy were in each case marked. The blood cholesterol was elevated in each of our animals fed with lanolin, increasing above normal to as high as 400 per cent.

We have, therefore, been unable to confirm Fahr, Van Leersum, Schmidtmann and Schönheimer, that feeding rabbits with cholesterol-containing substances, over long periods of time, causes elevations in blood pressure. The method used for the determination of the blood pressure is of course of the greatest importance. All three of the methods used by these investigators are open to serious criticism as has been pointed out above. For example, in the most direct of these methods, that of Van Leersum, alterations in the compressibility of the exposed carotid artery would be in favor of a higher blood pressure. By the method of Anderson, which we used, no surgical procedure is necessary, nor is it required to subject any part of the rabbit to unphysiologic conditions to obtain the readings.

The conception of atherosclerosis supported by Aschoff[8] and his pupils, especially Ranke,[9] is based on the older views of Virchow. This hypothesis assumes that certain mechanical influences and the processes of "wear and tear" play essential rôles. The primary anatomic change in the vessel wall, according to the Aschoff school, is a loosening of the intercellular cement substance of the intima and a deposition of fat and cholesterol esters within it. As the process continues, the deposition eventually involves all the layers of the intima, both cellular and interstitial elements.

The prevailing view, therefore, is that atherosclerosis is dependent upon certain mechanical factors, among which is increased vascular tension, which bring about loosening of the intimal cement substances and thus allows the deposition of fats and lipoids. Our observations on the blood pressure during the development of atherosclerosis in rabbits suggest that increase in blood pressure is not an essential factor in the deposition of lipoids in the intima.

CONCLUSIONS

1. The systolic blood pressure in the central artery of the rabbit's ear averages between 75 and 90 mm. mercury as reported by Anderson.

2. During the developmental stage of experimental lanolin atherosclerosis in rabbits, there is no significant hypertension.

3. It is believed that hypertension is not essential for the development of this type of atherosclerosis in rabbits.

We wish to thank Dr. David Marine for his helpful criticism in this work.

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FURTHER EXPERIENCES WITH THE VERNES TEST FOR THE DIAGNOSIS OF SYPHILIS*

By Leon H. Cornwall, M.D., New York

FOUR years ago, in a brief report, the work of Arthur Vernes which culminated in his new seroreaction for syphilis was reviewed by Cornwall and Aronson,[1] and in a subsequent communication[2] the results of 850 examinations of blood and spinal fluid from a series of controlled clinical cases were reported.

This test is based upon the observation that syphilitic serums when added in certain proportions to a colloidal solution produce flocculation, whereas normal serums do not. In 1917, Vernes published his observations concerning this phenomenon.[3] Since that time several methods for the diagnosis of syphilis based upon flocculation or precipitation have been reported, notably that of Sachs-Georgi in Germany (1918)[4] and Kahn in this country (1922).[5]

An alcoholic extract of horse heart is the colloid that is employed. In Vernes' first method, the flocculation was read indirectly by means of a hemolytic indicator consisting of sheep cells and pork serum. The details of this technic may be found in my publication of 1922.[2] Since that time the technic of the test has been modified twice, with a gain in simplicity each time.

The second method dispensed with the indirect method of attaining the result. Inactivated serum was added directly to the colloidal suspension (alcoholic extract of horse heart) in the proportion of two parts of serum to one part of the extract diluted 1 to 6.5. After incubation at 20° C. for four hours the turbidity of this mixture was determined with the photometer of Vernes and Bricq. A control tube containing the same amount and dilution of serum but without any colloidal suspension was treated in the same manner. The end-result was represented by the difference between these two readings. (Turbidity of serum + colloid − turbidity of serum only = result.)

The photometer of Vernes and Bricq was equipped with an arbitrary numerical scale. By making a large number of quantitative estimations of the weight of the flocculi in suspension, weight-volume equivalents were determined for the numbers of the photometric scale. By means of a key furnished with each instrument, therefore, the numerical readings could be translated into terms of milligrams of precipitate per cubic centimeter.

This second method was designated by Vernes as the "Mesure Ponderale de la Flocculation."[6] It was soon superceded, however, by a third method as a result of an improved photometer devised by Vernes, Bricq and Yvon in 1922.[7]

This new instrument made possible a rapid and precise measurement of

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light absorption (turbidity) and its direct expression in terms of optic density. The numbers of the scale correspond to degrees of optic density.

The results reported herein were obtained with the last method. The work has been done in the Neurological Department of the College of Physicians and Surgeons, Columbia University and was made possible by a grant from the Commonwealth Fund.

TECHNIC

1. Extract of Horse Heart (Perethynol).—The muscular part of a fresh horse heart is finely chopped or ground and the pulp obtained is dehydrated by maceration in 95 per cent alcohol. It is allowed to stand for about one hour, being occasionally stirred. At the end of that time the alcohol is partially expressed and the dehydration is terminated by thoroughly mixing the pulp and alcohol in a mortar. The alcohol is then removed by expression and the pieces of tissue are spread out in a thin layer on glass plates and dried

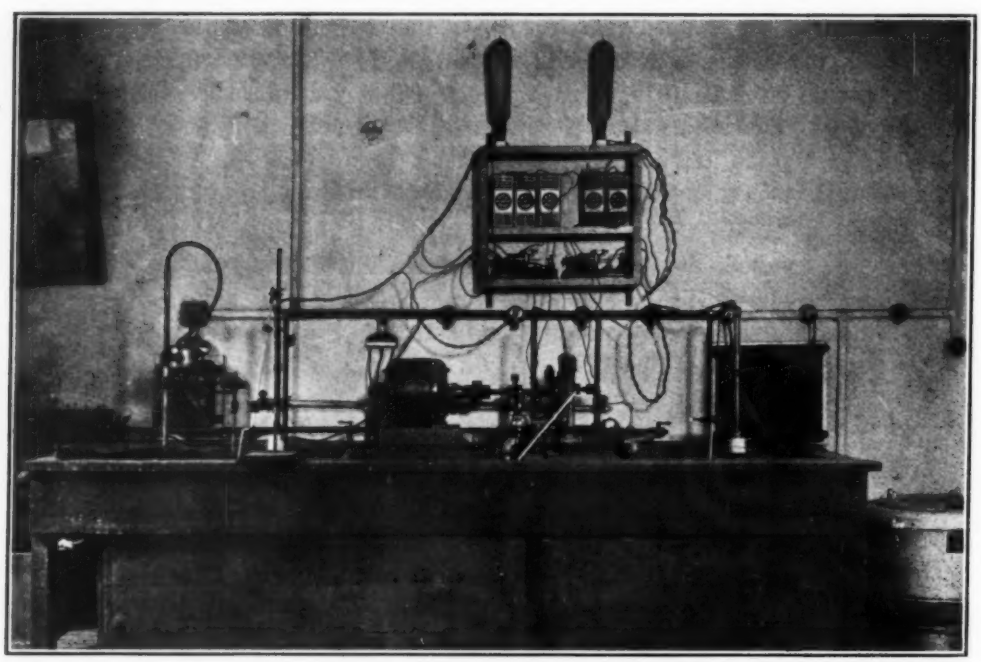

Fig. 1.—Water-baths built in a table. The stirring apparatus and delivery pipette are at the left. The centrifugal pump is in the middle background.

in the thermostat at 37° C. for twenty-four hours. The dried pulp is then finely pulverized in a mortar.

To 30 gm. of the powdered heart are added 60 gm. of sand, previously washed in alcohol and dried, and 250 c.c. of ethylene perchloride, the boiling point of which is from 115° to 121° C. This is placed in a 500 c.c. distilling flask which is connected with a Sohxlet extracting apparatus and condenser. The extraction apparatus is arranged so that the distillation may be conducted under partial vacuum by means of an air or a water pump. The distilling flask is heated in a water-bath, the temperature of which should be maintained at from 60° to 65° C. The operation is conducted at a pressure of 4 cm. of mercury so that the temperature in the distilling flask does not exceed 35° C. This requires from six to seven hours (forty siphonnements).

The powdered residue is again dried at 37° C. and to it are added 200 c.c. of absolute alcohol. Distillation is again accomplished by means of the same apparatus at a pressure

of from 5 to 6 cm. of mercury. The temperature of the water-bath should be from 60° to 65° C. and the temperature of the contents of the flask 30° C. The operation requires five hours (thirty siphonnements).

The residue is discarded and the final distillate is allowed to stand for twenty-four hours and then filtered. A small portion of this filtrate is dried at 60° C. for from eight to nine hours, and weighed. On the basis of this calculation, the alcoholic solution is adjusted so that it contains 15 gm. of the dried extract per liter. This is accomplished by the addition of more alcohol or by further evaporation in vacuum at 30° C. according to the indication.

2. Colloidal Suspension.—The extract of horse heart is diluted with doubly distilled water. If the extract is added, drop by drop, to the water, the latter being rapidly agitated during the process, the resulting suspension is pale and limpid because of the fine division of the molecules. If on the other hand distilled water is added, drop by drop to the extract, a milky suspension is obtained due to the large size of the molecules.

Because of the difference in the flocculent properties of blood serum and spinal fluid, colloidal suspensions of very different physical states are required. In each case the mix-

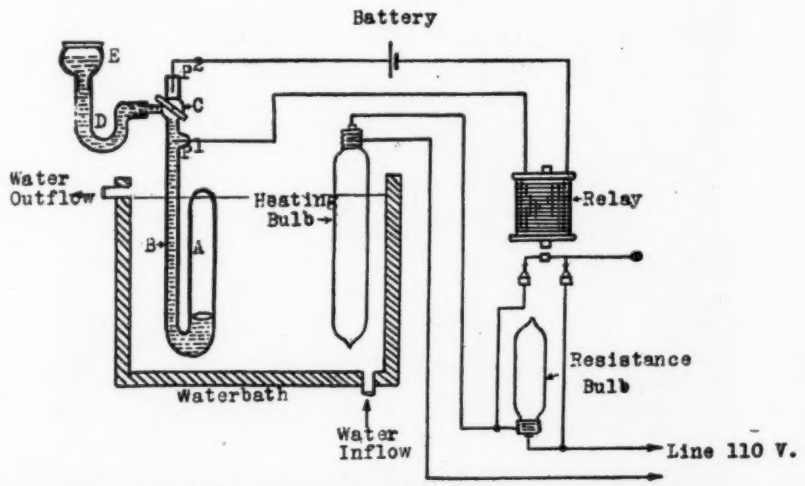

Fig. 2.—Diagram of water-bath with the electrical connections for heating and thermo regulation.

tures are made in a water-bath at the temperature of 25° C., all ingredients and glassware having been brought to this temperature before beginning the preparation of the suspension. During preparation, the ingredients are stirred by means of a mechanical apparatus having a glass propeller operated by an electric motor. The propeller is rotated at the rate of 200 revolutions per minute.

Preparation of Suspension, etc.

Vernes emphasized the necessity for uniformity of temperature during all stages of the procedure and his laboratory is kept at 20° C. It was impossible for us to accomplish this without constructing a thermostatic room which we were precluded from doing because of the expense.

It occurred to us that a water-bath of sufficient size for us to work in would meet the requirements, provided all tubes were immediately corked with rubber stoppers. In conferring with H. B. Williams of the Department of Physiology, we found that such a bath could be improvised without much difficulty or expense by adapting certain principles previously utilized by him in constructing a constant-temperature, water-cooled calorimeter. This improvised bath with the required attachments is illustrated in Figs. 1 and 2. The total cost did not exceed one hundred dollars.

It consists of a metal water tank jacketed with wood. There is an air chamber of approximately two inches between the metal tank and wooden jacket on the sides and bottom. This air chamber is filled with lagging for the purpose of insulation. Ground cork or felt in suitable material for lagging. A perforated metal shelf was inserted 1½ inches below the level of the water. The ingredients and test tube racks are kept on this shelf immersed in water of the proper temperature from the beginning to the end of the test. Constant circulation of water from the city supply is maintained in this tank by means of a Viking centrifugal pump. During the summer season, if the temperature of the tap water exceeds 20° C., it may be cooled by allowing it to pass through coils immersed in a freezing mixture of ice and salt. To obviate rapid changes in the temperature of the bath the tank should have a depth of 14 to 16 inches. The water is heated to the desired temperature by means of an electric heating lamp of 250 watts. The temperature is controlled by means of a chloroform mercury thermostat previously described by Williams[8]. This is illustrated in Fig. 2. It consists of a U-shaped glass tube with one closed bulbous

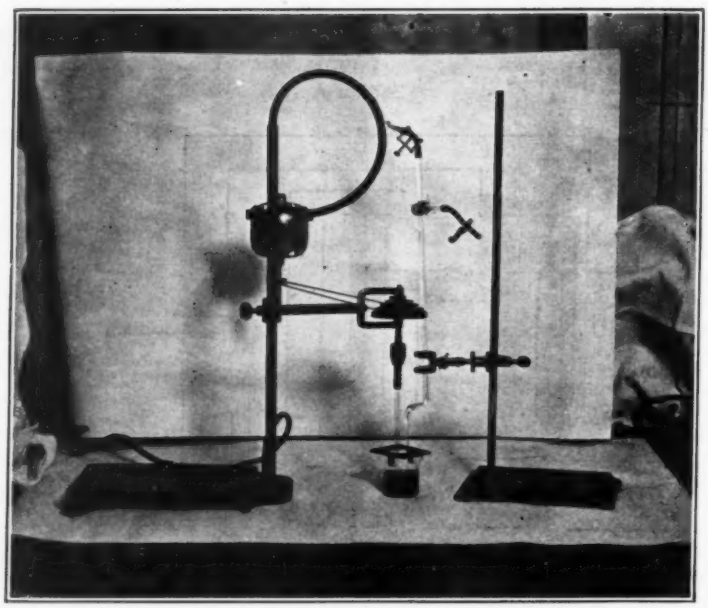

Fig. 3.—Stirring apparatus and delivery pipette employed for the preparation of the suspension.

arm, *A*. The other arm, *B*, is open and has a calibre of about 2 mm. Near the open end of *B* there is a three-way stopcock, *C*, and a lateral arm, *D*, communicating with the mercury reservoir, *E*. *A* is filled with chloroform and *B* with mercury from the reservoir. When the desired amount of mercury has been determined, communication between the reservoir, *E*, and *B* is closed by means of the three-way cock, *C*. A platinum wire, P^1, is fused into the side of the tube, *B*, and is always is contact with the mercury. Another platinum wire, P^2, projects into the upper open end of *B* through a small cork. The amount of mercury should be regulated so that, at the desired temperature, there is contact between it and the platinum tip, P^2, thus closing the relay circuit. When the relay is closed the heating and the resistance bulbs are in series, thus reducing the luminosity of the heating bulb. As soon as the mercury falls sufficiently to break its contact with P^2, the relay circuit is opened and the heating and resistance bulbs are parallel, causing the heating bulb to again become fully lighted. The low specific heat and high coefficient of cubical expansion of chloroform result in rapid changes in its volume with slight changes in the temperature of the water in the bath. This apparatus can be adjusted to maintain a constant temperature within a small fraction of a degree. We have found it extremely satisfactory.

Preparation of Suspension for Blood

Dilution 1/6.5

Place in a beaker of 45 mm. diam. doubly distilled water..... 12.0 c.c.
Add extract drop by drop at the rate of 1 c.c. per minute..... 6.0 c.c.
Add doubly distilled water ..... 21.0 c.c.

Diameter of propeller 35 mm. The tip of delivery pipette should be about 3 cm. from surface of liquid.

Preparation of Suspension for Spinal Fluid

Dilution 1/4

Place in a beaker of 28-30 mm. diam. extract..... 3.0 c.c.
Add doubly distilled water, drop by drop at the rate of 3 c.c. per minute.....9.0 c.c.

After 1/3 of the water has been added the remainder may be added more rapidly. Diameter of propeller 15 mm. The tip of delivery pipette should be in contact with surface of liquid.

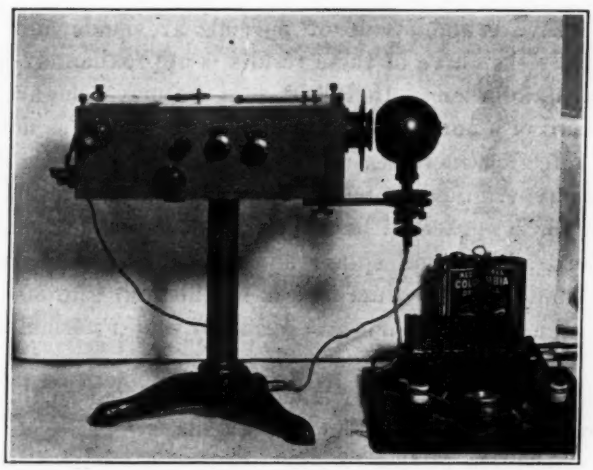

Fig. 4.—Photometer of Vernes, Bricq and Yvon.

3. TEST.—Tubes of 60 by 13 mm. dimensions are used.

Blood

	(Test) Tube I	(Control) Tube II
	c.c.	c.c.
Serum-inactivated 20 minutes at 55° C.	0.8	0.8
Extract (Perethynol) 1/6.5	0.4	0.0
Absolute alcohol 1/6.5	0.0	0.4

Cork with rubber stoppers, shake, and place in water-bath for four hours at 25° C.
Result: Optic density of Tube I minus Tube II.

Spinal Fluid

	Tube I (Test)	Tube II (Control)
	c.c.	c.c.
Spinal fluid	1.6	0.0
Extract (Perethynol) 1/4	0.4	0.4
Doubly distilled water	0.0	1.6

Five control tubes are prepared for each series of spinal fluid tests. The average optic density of these five tubes is taken as a control for the whole series. The tubes are corked with rubber stoppers, shaken and placed in the water-bath at 25° C. for four hours.

The optic density of turbid spinal fluid is read immediately and this result is subtracted from the final reading.

4. FINAL RESULT.—After incubation for four hours the tubes are inverted several times and the optic density of Tubes I and II determined. The optic density of Tube I minus the optic density of Tube II gives the result.

Before presenting the results of this reaction, it is necessary to explain the method that must be applied for its interpretation.

First, all blood serums and spinal fluids produce flocculation or precipitation under certain conditions.

Second, the precipitation caused by normal serum varies in different individuals just as the normal temperature or pulse rate varies in individual cases.

Third, the average mean flocculence of normal blood serum and spinal fluid has been established and the upper limit of flocculence in normals has been determined.

When successive examinations of normals are made and the results are charted graphically the curve of these results is approximately a horizontal line with only slight undulations. In this respect we may compare the graph to a temperature record of a normal individual. This is known as normal flocculence.

Syphilitic infection increases the flocculation. Syphilitic serum is spoken of, therefore, as hyperflocculent or surflocculent and the result of the reaction obtained therewith is spoken of as hyperflocculence or surflocculence.

Normal blood serums and spinal fluids show oscillations that are usually within narrow limits (blood serum 0 to 6, spinal fluid 0 to 30). In rare cases, the flocculence of a normal serum may reach 27 and a normal spinal fluid may reach 50.

The sensitiveness of the reaction has been adjusted so that normal flocculence is confined to the lower degrees of the scale.

A proper conception of this reaction requires a modification of the terminology that we have applied to serologic reactions.

Antigen is a biologic term signifying a substance that stimulates the formation of antibodies in the host. The extract used for this test is not an antigen. We may call it reagin if we choose. In reality it is a colloidal suspension possessing certain definite physicochemic properties. The reaction produced by the mixture of this suspension with blood serum or spinal fluid is a chemical or physicochemic one viz., flocculation or precipitation. This flocculation or precipitation is accompanied by a modification of the turbidity of the mixture. This modification of turbidity is expressed in terms of optic density, a factor that has a definite and well understood physical significance.

Optic density is a measure of light absorption or diffusion (logarithm of the incident minus the transmitted light). It is determined by means of the photometer previously alluded to.

In expressing the results of this reaction we must discard the terms "negative," "doubtful," "weakly positive," "positive," "strongly positive," etc., and substitute numerical terms of optic density. Failure to do this will retard the understanding of the reaction and defeat the object of its use. Except in rare instances it cannot furnish a diagnosis when the result is divorced from the clinical aspects of the case to which it is applied.

The reaction is designed to be correlated with clinical findings. The interpretation should be made by one who is in touch with all of the essential clinical facts.

In truth, no laboratory procedure is designed to diagnose, in disregard of or in ignorance of, clinical facts although unfortunately the laboratory is too frequently called upon to serve this function.

It should be emphasized that the interpretation of this procedure depends upon the curve obtained by successive examinations of the blood serum or spinal fluid. Its diagnostic significance depends upon the correlation of the results with clinical observations.

For example, a group of clinical facts present themselves in a given case that suggest the possibility of syphilitic etiology although no history of syphilitic infection is given nor are there any physical signs that give reasonable assurance of this. The Vernes result shows an optic density of zero. Can one postulate the absence of syphilis? No. No more surely than one can postulate the absence of hyperthermia with one record of a temperature of 98.6° F. One can only say that the flocculation in one case and the temperature in the other were within the range of normal at that particular time.

Most nonsyphilitic serums give a photometric reading of 0 to 6. An elevation to 27 in a presumably nonsyphilitic serum has been observed only once by Vernes in over 650,000 examinations. The reaction has been so regulated that the vast majority of nonsyphilitic serums give readings from 0 to 6 or 8 and the oscillation noted on successive examinations is usually confined within these limits. This characteristic is distinctive for nonsyphilitic serums. One can speak of complete absence of flocculation only when the photometric result is 0.

Results from 0 to 8 indicate the absence of syphilis in a vast majority of cases.

The original technic for spinal fluid was so regulated that nonsyphilitic fluids gave photometric readings as high as 30 and at least 50 was necessary to justify a diagnosis of meningeal syphilis on a single examination. Subsequent modification of the technic has provided the same limits for spinal fluids as for blood. *Our results were obtained with the earlier technic.*

As a therapeutic index, this test offers features of especial attraction. The graph of successive results displays, in an objective manner not possessed by other serologic reactions, the efficacy of the therapy upon the surfloccule nce. The photometric readings may descend to the nonsyphilitic zone but if the treatment has been insufficient, the curve will rise again.

The rise or fall or stability of the photometric readings indicates the progression, regression, or stationary character of the infection.

One more important point must be mentioned. This concerns the significance of a low photometric reading in a case of treated syphilis. Does this mean that the infection has been extinguished? In such a case Vernes recommends the administration of one or two doses of an arsenical. Following this, he advises monthly examinations of the blood for eight months and an examination of the spinal fluid at the eighth month. If the curve does not oscillate beyond the limits prescribed for nonsyphilitic serums during that period and the spinal fluid is negative at the eighth month, he states that he has never seen

a recurrence of the surflocculence in his experience which includes over 650,000 results. In case the infection has not been extinguished, the administration of arsenic will have a provocative action on the flocculation. Low flocculence or the absence of flocculence for eight months is not alone sufficient. This period must follow arsenical administration. This is spoken of as the "jalon arsenical," or postarsenical interval.

We have collected our records of the application of the Vernes and Wassermann tests on 1466 blood serums and spinal fluids from cases in the Neurological Department of the Vanderbilt Clinic. Our purpose was to make a comparative study of the relative interpretative value of the two tests rather than merely a numerical comparison of the results of the two tests.

Histories and clinical data have been carefully investigated in arranging the clinical groups. It would not have been difficult to multiply the number of results herein contained but mere numbers would fail to give an idea of the relative value of these tests for clinical application.

BLOODS

Twelve hundred and ninety-seven serums were examined.

The cases were divided into three clinical groups: nonsyphilitic, syphilitic, and miscellaneous.

I. Nonsyphilitic Group

The serums numbered 758.

The Wassermann reactions were negative in 735 and indeterminate in 23.

In the twenty-three serums that gave indeterminate Wassermann reactions the photometric readings were 0 to 7. Careful investigation of these cases failed to disclose anything suggestive of syphilis.

The Vernes photometric results were as follows:

0 to 4	in	634 serums
5 to 9	in	90 serums
10 to 20	in	31 serums
21 to 24	in	3 serums
		758 serums

Of the three cases giving photometric readings above 20, two were psychoneurotics and one a mental defective. The thirty-one serums that gave photometric readings from 10 to 20 came from cases diagnosed as follows (page 839):

Only a very few serums in this group were examined more than once. In these, the photometric variation was from 0 to 3 points.

In this group of 758 serums from clinically nonsyphilitic cases, most of which were examined once only, the Wassermann result was indeterminate 23 times. None of the Vernes readings were such as to enable one to affirm syphilis. The serums that gave photometric readings above 10 (thirty-four in number) presumably represent a group that possess high flocculence normally.

DIAGNOSIS	NO.	PHOTOMETRIC READING
Alcoholism	1	13
Arteriosclerosis	1	15
Cephalalgia	1	10
Epilepsy	1	11
	1	13
	1	15
Facial neuralgia	1	14
Frontal sinusitis	1	18
Hemiplegia	1	17
Myasthenia	1	12
Myositis	1	12
Myxedema	1	11
Neuritis (traumatic of ulnar)	1	13
Psychoneurosis	1	19
	1	18
	1	16
	3	15
	3	13
	2	12
	4	10
Speech Defect	1	12
Sydenham's chorea	1	16
Taenia circinata	1	14

TABLE I

SHOWING COMPARATIVE WASSERMANN AND VERNES RESULTS ON 758 BLOOD SERUMS OF NONSYPHILITICS

NO. SERUMS	WASSERMANN RESULTS	VERNES RESULTS
701	Negative	0 to 9
31	Negative	10 to 20
3	Negative	21 to 24
23	Indeterminate	0 to 7
758		

II. Syphilitic Group

Five hundred and twenty serums were examined from cases of known syphilis. They were subdivided as follows:

1. Neurosyphilis	366
2. Congenital syphilis	14
3. Vascular syphilis	7
4. Latent syphilis (Treated and untreated)	133
	520

Most of these cases were under treatment at our clinic or had received treatment elsewhere. Repeated serologic examinations were made, in some instances ten or more; therefore, we were able to study the oscillations of the photometric results from graphs.

Space does not permit a detailed record of these results and a comprehensive idea of the comparison of the two tests cannot be shown in a condensed tabulation. A proper interpretation of the significance of Vernes results requires a knowledge of the amount and periodicity of treatment as well as the intervals between the successive tests. These features can be seen by a glance at the charts.

CASE 1.—J. A., admitted July, 1919, male, age 32. Syphilis contracted 1915. Treatment begun immediately.

Complaints.—Nervousness, irritability, precordial pain, weakness.

Neurologic Examination.—Negative.

Blood Wassermann: ++++.

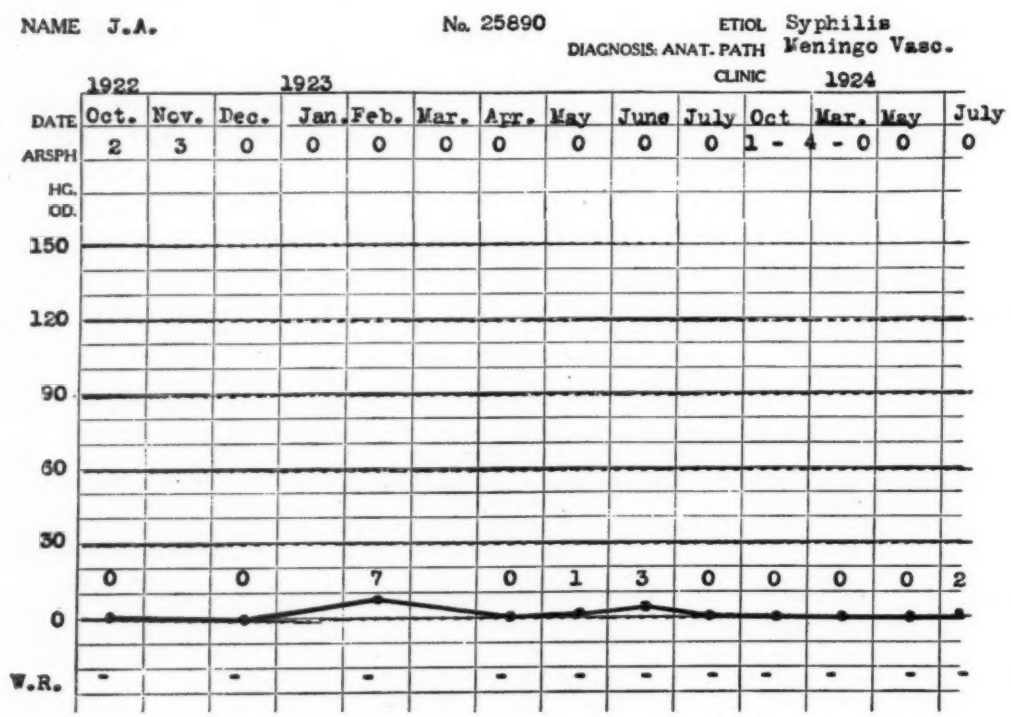
NAME J.A.
No. 25890
ETIOL Syphilis
DIAGNOSIS. ANAT. PATH Meningo Vasc.
CLINIC
1922
1923
1924
DATE Oct. Nov. Dec. Jan. Feb. Mar. Apr. May June July Oct Mar. May July
ARSPH 2 3 0 0 0 0 0 0 0 0 1 - 4 - 0 0 0
HG.
OD.
150
120
90
60
30
0 0 7 0 1 3 0 0 0 0 2
0
W.R. - - - - - - - - - - -

Chart I.

Spinal Fluid: Cells 6, globulin +, Wassermann ++++ in 0.2 c.c.; Lange 44332210000.

Diagnosis.—Meningovascular neurosyphilis.

Treatment July, 1919, to March, 1921: 28 salvarsan injections intravenously; 14 mercury injections intramuscularly, 9 intraspinal injections—salvarsanized serum. Spinal fluid became negative July, 1920. Blood fluid became negative March, 1921.

This case has shown a negative Wassermann reaction on eight occasions during a period of 17 months. The Vernes has shown an oscillation of only 7 points during this period. We believe that the spirochetosis has been eradicated. See Chart I.

CASE 2.—A. A., admitted July, 1919, female, age 25, wife of patient reported as Case 1. Positive Wassermann discovered 1917, after a miscarriage.

Complaints.—Loss of weight, weakness, dizziness, pains in legs.

Neurologic Examination.—Negative except for tremor of hands and inequality of patellar reflexes.

Blood Wassermann: ++++.

Spinal Fluid: Cells 0, globulin normal, Wassermann ++++ in 0.8 c.c.; Lange 1233210000.

Diagnosis.—Meningovascular neurosyphilis (conjugal).

Treatment August, 1919, to June, 1922: 24 salvarsan injections intravenously; 5 mercury injections intramuscularly; 7 intraspinal injections—salvarsanized serum.

Blood became negative in June, 1922. Spinal fluid became negative in all respects except Lange 4422231000 in June, 1920.

The Wassermann reaction between April, 1923, and May, 1924, varied from one-plus to doubtful. The Vernes curve shows an oscillation of greater ampli-

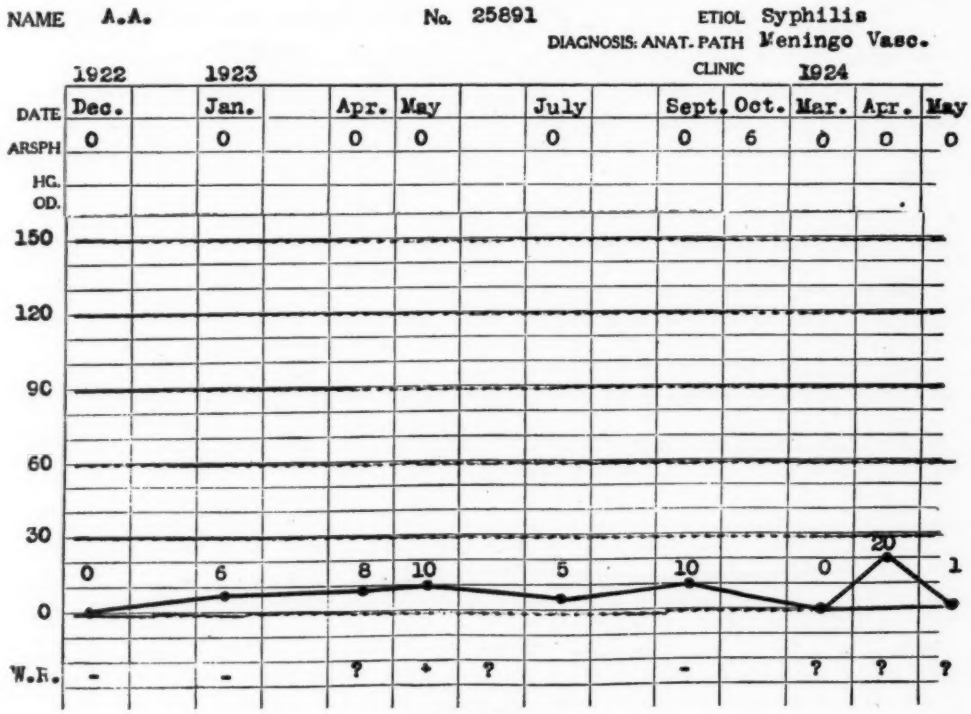

Chart II.

tude than one obtains with nonsyphilitic serum. The low points of 0 show that the normal flocculence in this case was not high. Note that in April, 1924, the curve rose to 20, whereas, the Wassermann remained unchanged. The single results would not be especially significant but the curve is illuminating. The infection is not controlled and further treatment is indicated. See Chart II.

CASE 3.—B., admitted November, 1920, female, age 54. Conjugal syphilitic infection, 1902. Husband a tabetic.

Complaints.—Insomnia, tinnitis aurium, anxiety, diplopia.

Neurologic Examination.—Left ptosis, slight ataxia, pupillary inequalities with Argyll Robertson on the right side, diminution of both corneal reflexes.

Blood Wassermann: ++++.

Spinal Fluid: Cells 9, globulin trace, Wassermann ++ in 1.0 c.c.; Lange 4424332211.

Diagnosis.—Meningovascular neurosyphilis.

Treatment from November, 1920, to October, 1922: 65 salvarsan injections intravenously; 34 mercury injections intramusculary; 5 intraspinal injections—salvarsanized serum. Blood first became negative in June, 1921. Remained so until January, 1922, when it was +. Spinal fluid in April, 1921: Cells 3, globulin trace, Wassermann: Negative in 2.0 c.c.; Lange 5555543210.

In so far as one can judge clinically, the infection has been arrested in this case. It will be noted from Chart III that there is a considerable inconsistency in the Wassermann and Vernes results. In comment, I can say that with assurance that the Vernes results tally more accurately with the clinical aspects of the case than do the Wassermann results. See Chart III.

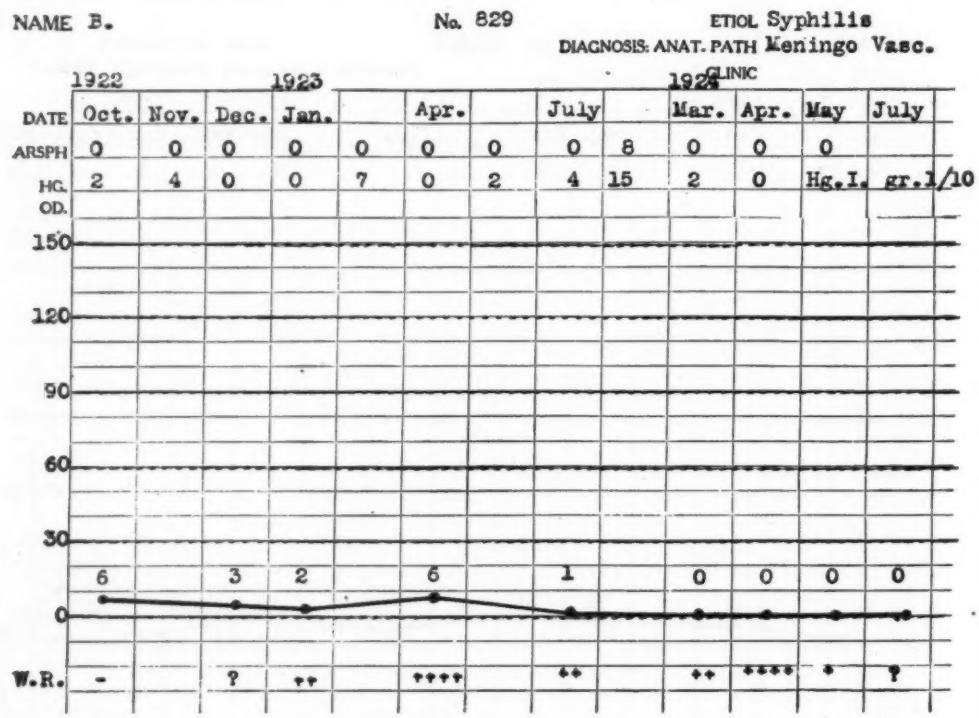
NAME B.
No. 829
ETIOL Syphilis
DIAGNOSIS: ANAT. PATH Meningo Vasc.
CLINIC
1922
1923
1924
DATE Oct. Nov. Dec. Jan. Apr. July Mar. Apr. May July
ARSPH 0 0 0 0 0 0 0 0 8 0 0 0
HG. 2 4 0 0 7 0 2 4 15 2 0 Hg.I. gr.1/10
OD.
150
120
90
60
30
6 3 2 6 1 0 0 0 0
0
W.R. - ? ++ ++++ ++ ++ ++++ + ?

Chart III.

CASE 4.—B., admitted January, 1923, male, age 41. Syphilis admitted. Date of infection unknown.

Complaints.—Gastric crises, shooting pains in legs and shoulders.

Previous Treatment.—30 salvarsan injections intravenously; 40 mercury injections intramuscularly; 2 intraspinal injections—salvarsanized serum.

Neurologic Examination.—Slight swaying in Romberg, tremor of hands and tongue, Argyll Robertson pupils, right facial paresis.

Blood Wassermann: +++.

Spinal Fluid: Cells 0, globulin normal, Wassermann +++ in 1.0 c.c.; Lange 1111000000.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis, partial tabetic syndrome.

Treatment from January, 1923, to April, 1923: 7 salvarsan injections intravenously; 27 mercury injections intramuscularly; 3 intraspinal injections—salvarsanized serum. At the end of that time the blood was doubtful and the spinal fluid essentially unchanged.

Note the oscillation of seventeen points on the Vernes curve between April and July, during which time no salvarsan was administered. When the Vernes was highest the Wassermann was negative. See Chart IV.

CASE 5.—C. B., admitted January, 1920, male, age 41. Syphilis admitted. Date of infection unknown.

Complaints.—Painless swelling of left ankle (Charcot's joint) numbness of both feet, ankles, finger tips, and left leg, girdle sensation.

Neurologic Examination.—Tremor of fingers, ataxia, atrophy of left calf muscles, absent patellar reflexes, inequality of pupils with sluggish reaction to light on the left side.

Blood Wassermann: ++++.

Spinal Fluid: Cells 19, globulin ++, Wassermann ++++ in 0.5 c.c.; Lange 5554332100.

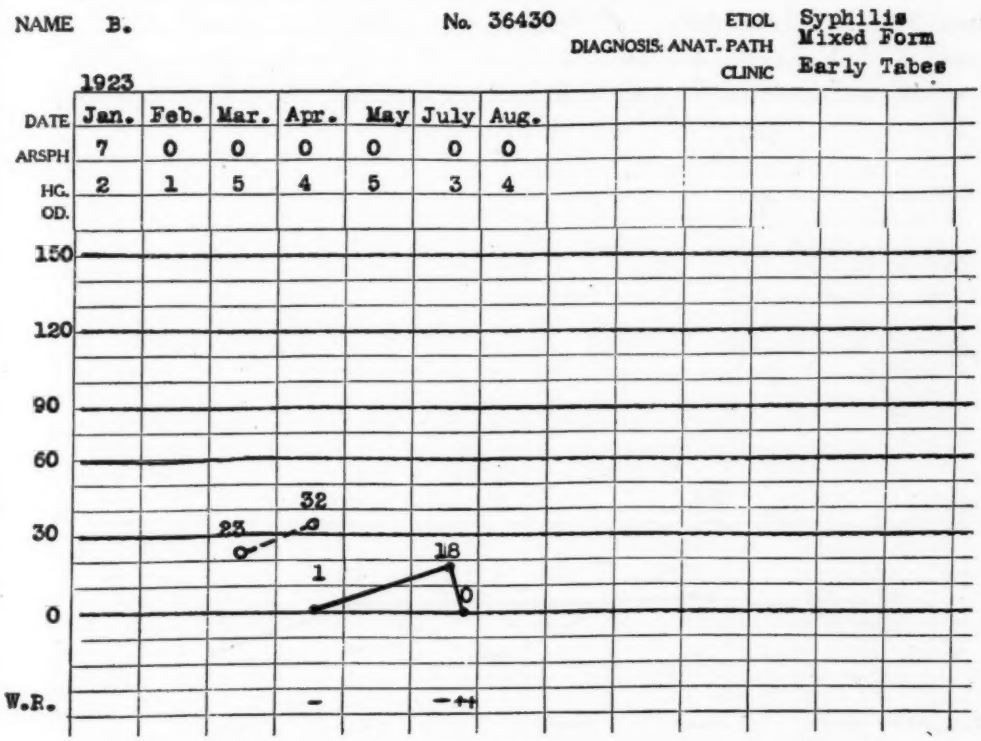

Chart IV.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis of encephalospinal distribution.

Treatment from January, 1920, to October, 1922: 40 salvarsan injections intravenously; 59 mercury injections intramuscularly; 22 intraspinal injections—salvarsanized serum.

This was a case of deeply seated neurosyphilis. The foci of infection were resistant to the action of spirocheticides probably on account of the failure of the drugs to penetrate. Although both reactions were positive in blood and spinal fluid, the Vernes indicates the changing character or intensity of the infection from time to time. It would appear that this feature of the Vernes test should be a valuable guide for the determination of the most efficient dosage and periodicity. A few doses of arsenicals properly spaced may be the means of arresting or overcoming an infection that otherwise would progress. In this

respect the conquest of syphilis might be compared to a hurdle race, which is only won after the last hurdle is cleared. If the runner should fail at the last obstacle the energy expended up to that point goes for naught and his race is lost. So in the treatment of syphilis, energetic treatment must be pursued till the last barrier is penetrated provided such a result is possible. If the Vernes curve is descending, treatment should be so adjusted as to favor the continuation of the descent to its base line. See Chart V.

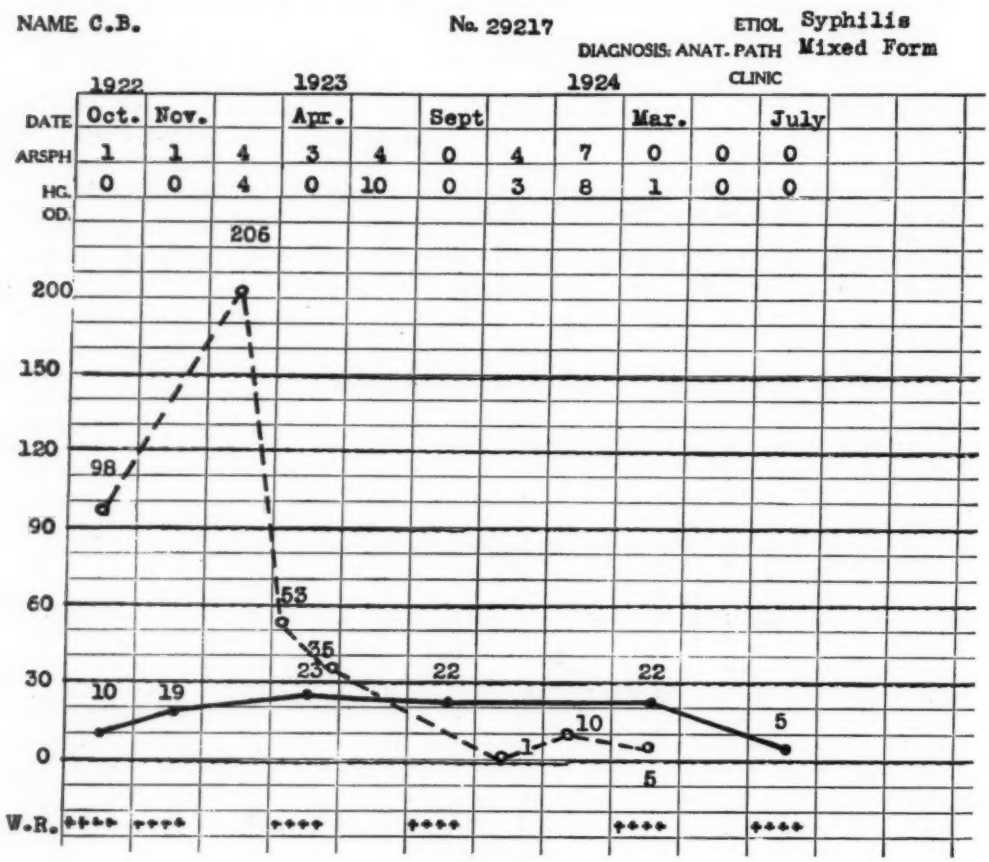

Chart V.

CASE 6.—C. L., admitted November, 1918, female, age 43. Probable conjugal infection. Duration of symptoms seven years.

Complaints.—Loss of weight, pain in back, and failing memory.

Neurologic Examination.—Romberg positive, absent patellar reflexes, absent right Achilles reflex, inequality of pupils and sluggishness of reaction to light.

Blood Wassermann: ++++.

Spinal Fluid: Cells 23, globulin ++++, Wessermann ++++ in 0.4 c.c.; Lange 1123210000.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis. Partial tabetic syndrome.

Treatment from November, 1918, to March, 1923: 14 salvarsan injections intravenously; 52 mercury injections intramuscularly; 9 intraspinal injections—salvarsanized serum. Blood Wassermann persistently ++++. Spinal fluid became negative in January, 1921.

Unfortunately this patient is intolerant to arsenic, reacting with severe dermatitis to small amounts. Therefore, mercury, bismuth, and the iodides have had to be employed. The resistant foci are probably in the walls of the blood vessels. See Chart VI.

CASE 7.—L. P., admitted October, 1918, male, age 41. Infection contracted in 1915.

Complaints.—Shooting pains in legs, bladder disturbances, failing vision, diplopia.

Neurologic Examination.—Positive Romberg, coarse regular tremor of both hands, inequality of deep reflexes, inequality of pupils, bilateral Argyll Robertson pupils.

Blood Wassermann: ++++.

Spinal Fluid: Cells 20, globulin ++, Wassermann ++++ in 0.2 c.c.; Lange 5555443000.

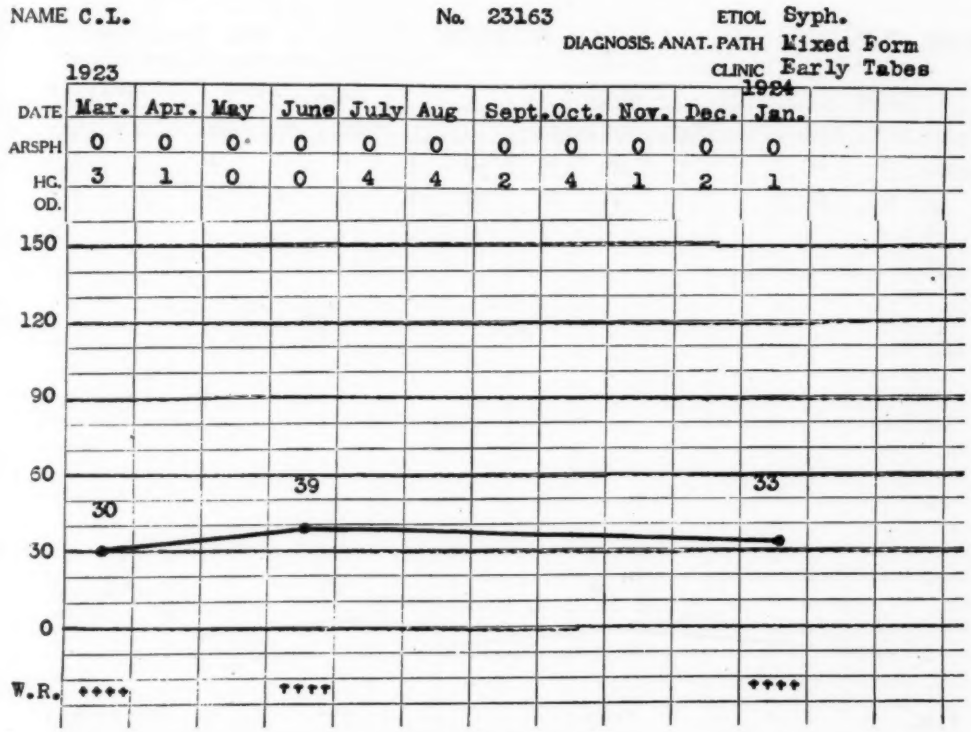

Chart VI.

Diagnosis.—Mixed mesodermal and ectodermal neurosyphilis with syphilitic encephalitis.

Treatment October, 1918, to October, 1922: 67 salvarsan injections intravenously; 113 mercury injections intramuscularly; 38 intraspinal injections—salvarsanized serum.

After treatment.—Blood Wassermann persistently ++++, spinal fluid unchanged.

Treatment in this case has been pursued over a period of six years. The neural pathology is believed to involve meninges, blood vessels and parenchyma. An episode occurred, in 1922, typical, in its psychic and somatic manifestations, of general paresis. By persistent treatment with only short and widely separated periods of rest the economic efficiency of the patient has been preserved so as to enable him to successfully conduct a business that furnishes a livelihood to his family of five and a staff of ten or more employees. See Chart VII.

CASE 8.—G. W., admitted June 9, 1922, male, age 41. Syphilitic infection twenty years previous.

Complaints.—Pain in back and legs, unsteady gait.

Neurologic Examination.—Ataxia, thick and hesitating speech, coarse tremor of fingers, absent patellar reflexes, sluggish pupillary reaction to light.

Blood Wassermann: +++.

Spinal Fluid: Cells 25, globulin ++, Wassermann +++ in 1.0 c.c.; Lange 2333210000.

Diagnosis.—Ectodermal neurosyphilis, tabes dorsalis.

Treatment previous to October, 1922: 15 salvarsan injections intravenously; 15 mercury injections intramuscularly; 6 intraspinal injections—salvarsanized serum. Blood Wassermann became negative after 3 salvarsan injections. Spinal fluid became negative in May, 1923.

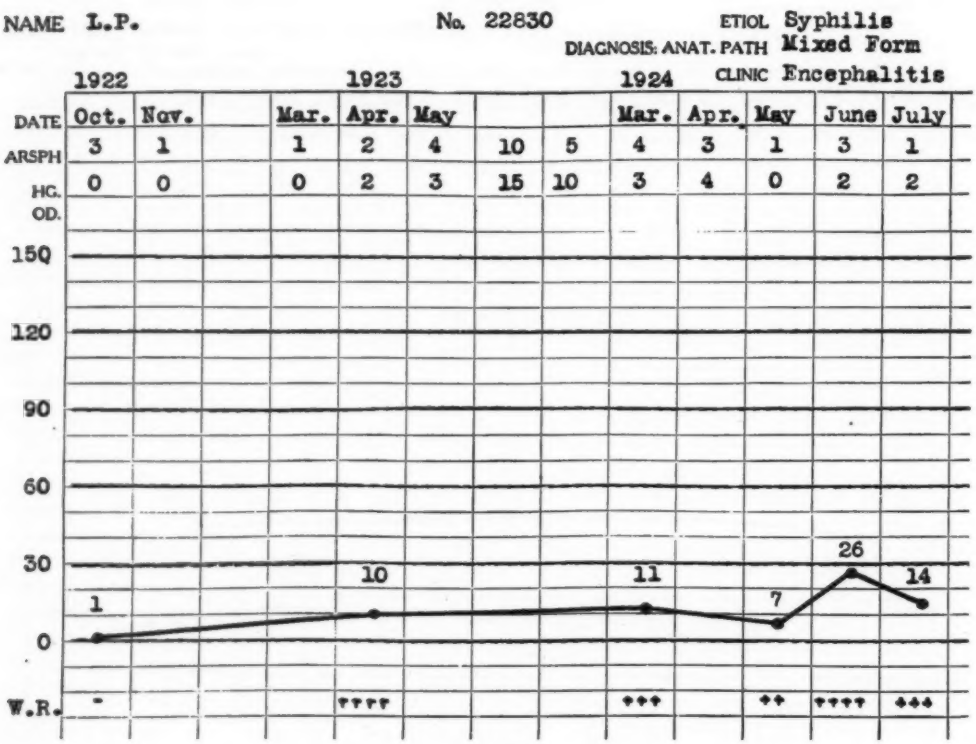

Chart VII.

This case exemplifies the clinical arrest of a tabetic syndrome that began with severe lightning pains and locomotor difficulty. The Vernes curve ascended 13 points while the Wassermann remained negative or doubtful. See Chart VIII.

CASE 9.—T. H., admitted March, 1923, male, age 37. Syphilis denied.

Complaints.—Insomnia, lack of ambition, lack of ability to concentrate.

Neurologic Examination.—Tremor of hands, hyperactive deep reflexes.

Blood Wassermann: ++++.

Spinal Fluid: Cells 0, globulin negative, Wassermann negative; Lange 1111100000.

Diagnosis.—Vascular type of mesodermal neurosyphilis with anxiety psychoneurosis.

An illustration is here shown of the necessity of successive Vernes reactions in order to establish a curve. The results considered individually have

but little significance, but the oscillation (descent) of 14 points during four months of treatment and the subsequent ascent of 10 points during which period the Wassermann remained practically stationary illustrates the comparative significance of the two tests. (Chart IX.)

CASE 10.—A. J., admitted May, 1923, male, age 50. Syphilitic infection denied.
Complaints.—Insomnia, precordial pain, palpitation of the heart, anxiety.
Neurologic Examination.—Fine tremor of both hands, thick speech, otherwise negative.
Blood Wassermann: +++.
Spinal Fluid: Cells 8, globulin +, Wassermann ++++ in 0.2 c.c.; Lange 1122210000.
Diagnosis.—Meningovascular neurosyphilis with anxiety psychoneurosis.

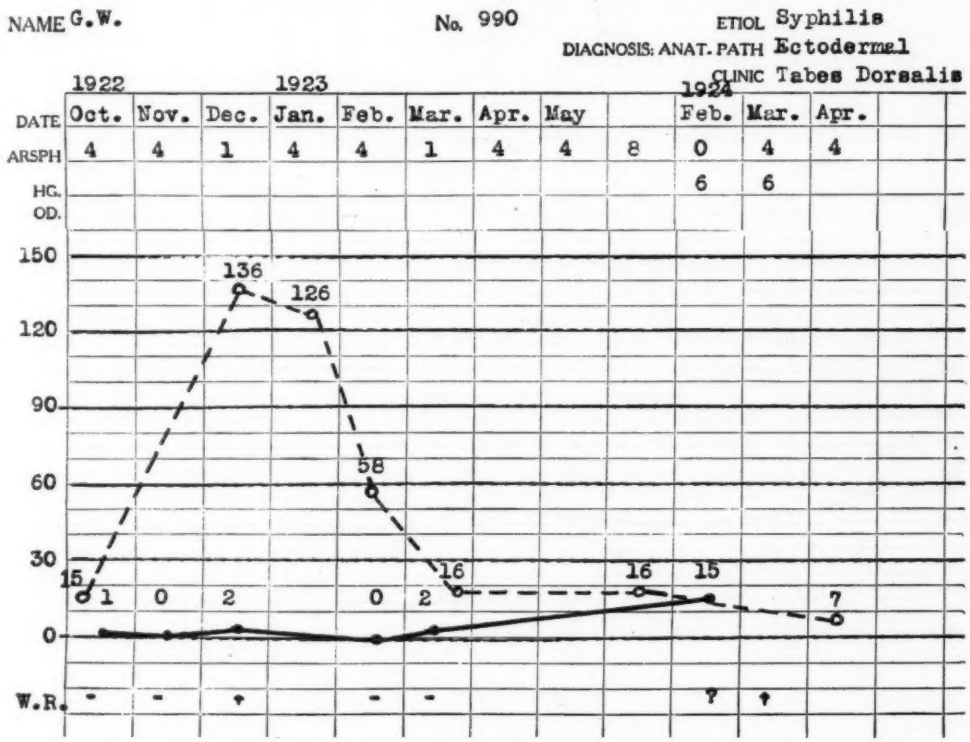

Chart VIII.

The comparative flexibility of the two tests may be seen. While the Wassermann remained four-plus the Vernes oscillated between 0 and 40. After reaching 0 the Vernes maintained this base line but the Wassermann fluctuated. See Chart X.

CASE 11.—F. R., admitted May, 1919, male, age 53. Syphilitic infection denied.
Complaints.—Pains in shoulders and back, girdle sensation.
Neurologic Examination.—Negative except for hyperactive patellar reflexes.
Blood Wassermann: ++++.
Spinal Fluid: Cells 4, globulin +, Wassermann negative in 2.0 c.c.; Lange 0000000000.
Diagnosis.—Vascular neurosyphilis, syphilitic aortitis.
Treatment from May, 1919, to October, 1922: 23 salvarsan injections intravenously; 58 mercury injections intramuscularly.

This case has been considered Wassermann fast. The Vernes oscillation confirms the Wassermann but its low character suggests that properly spaced treatment would be effective in permanently obliterating this oscillation. In such a case with divergent results of these two serologic tests, clinical facts must determine the relative value of the two findings. In this particular case the medical features consisted of myocarditis, aortitis, and the vascular type of kidney disease. See Chart XI.

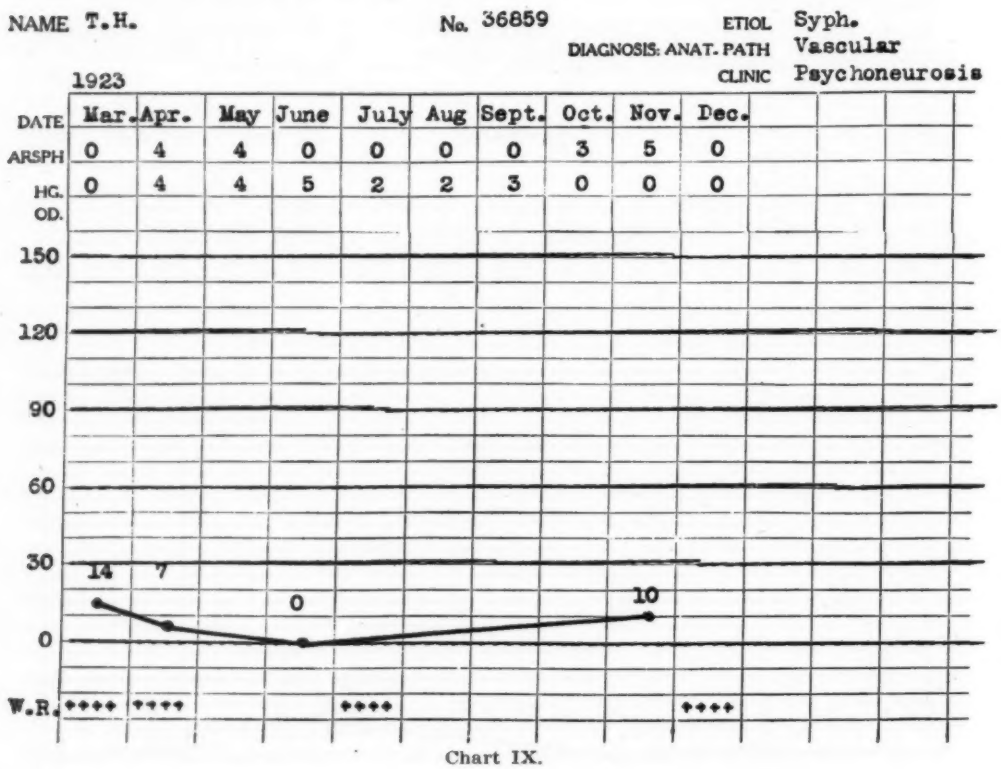

Chart IX.

MISCELLANEOUS GROUP

Nineteen serums were included in this group comprising cases concerning which there was reasonable uncertainty. The histories and clinical examinations did not warrant placing them in any of the other groups. Some denied syphilis but had positive Wassermann reactions or hyperflocculence, others said that they had had syphilis but it was not confirmed clinically or serologically. The whole group requires further study.

Three cases were diagnosed clinically as possible neurosyphilis. There was no history of infection. Wassermann reactions were negative and the Vernes results were 5, 3, and 0.

Five other serums were negative to the Wassermann reaction but four of these gave Vernes results requiring further study before a definite opinion was warranted and the fifth gave a Vernes result of 76 definitely affirming syphilis.

One case admitted syphilis but clinically presented only psychoneurotic symptoms. The Wassermann was four-plus and the Vernes 7.

Two cases were the wives of syphilitics whose serology was done as a precautionary measure. They presented no symptoms. The Vernes results were 8 and 69 and the Wassermann reactions of both were four-plus.

There were two cases of general arteriosclerosis and hypertension with no history of syphilis. In one, the Wassermann was two-plus and Vernes 0; in the other, the Wassermann was four-plus and the Vernes 0.

One case with right facial paresis admitted syphilis. The Wassermann was four-plus and the Vernes 1.

One case of myocarditis denied syphilis but had a four-plus Wassermann and Vernes of 66.

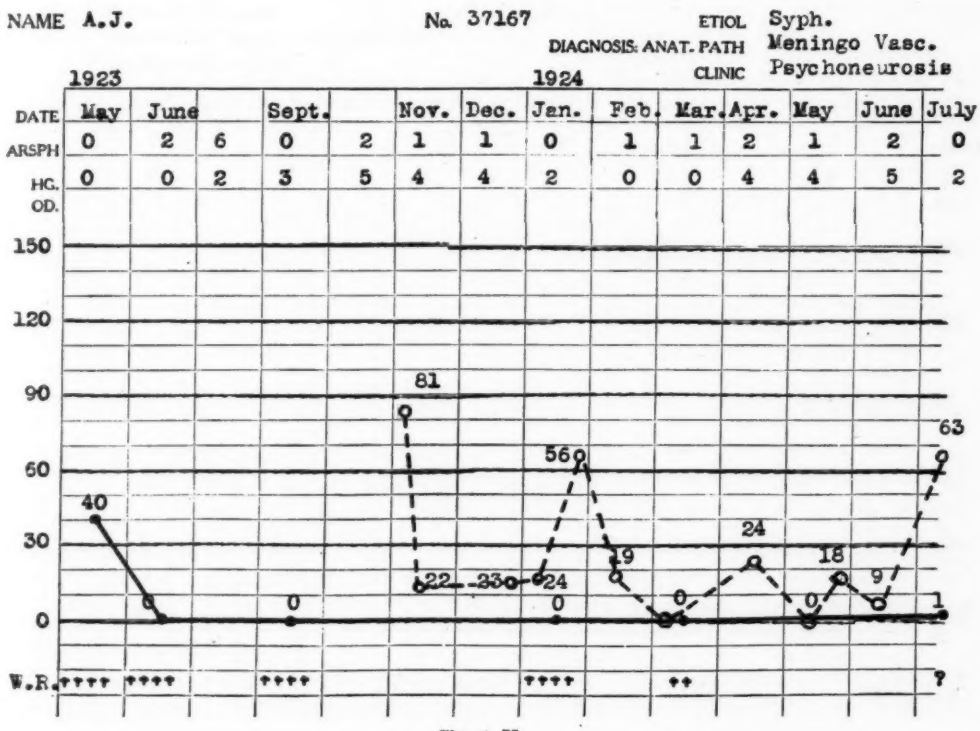

Chart X.

Four cases that denied syphilis had positive Wassermann serology and Vernes results of 7, 5, 13, and 5 respectively.

From a clinical standpoint, ten of the cases included in this group were considered as probably syphilitic (1, 2, 3, 5, 7, 8, 9, 10, 11, 14). The Wassermann results supported this conclusion in four cases (9, 10, 11, 14). Single Vernes results were confirmatory of syphilis in two cases (5 and 11) and in four cases (7, 8, 9, and 10) were insufficient to affirm or deny syphilis without further investigation.

In eight cases (4, 6, 12, 13, 16, 17, 18, 19) syphilitic infection was denied and clinically they were regarded as nonsyphilitic. The Wassermann was negative in two (4 and 6), four-plus in two (13 and 19), two-plus in three (12, 17, 18) and one-plus in one (16). Single Vernes results indicated probable

syphilis in two cases (4 and 6) in which the Wassermann was negative and suggested the necessity of further investigation in one case (18) in which the Wassermann was two-plus. The other five Vernes results, taken alone, signified only that there was no hyperflocculence at the moment of examination.

One case of myocarditis (15) in which syphilis was denied gave Wassermann and Vernes results confirmatory of syphilis—four-plus and 66.

SPINAL FLUIDS

Up to October, 1923, one hundred and sixty-nine spinal fluids from sixty-two cases of neurosyphilis were submitted to both Wassermann and Vernes

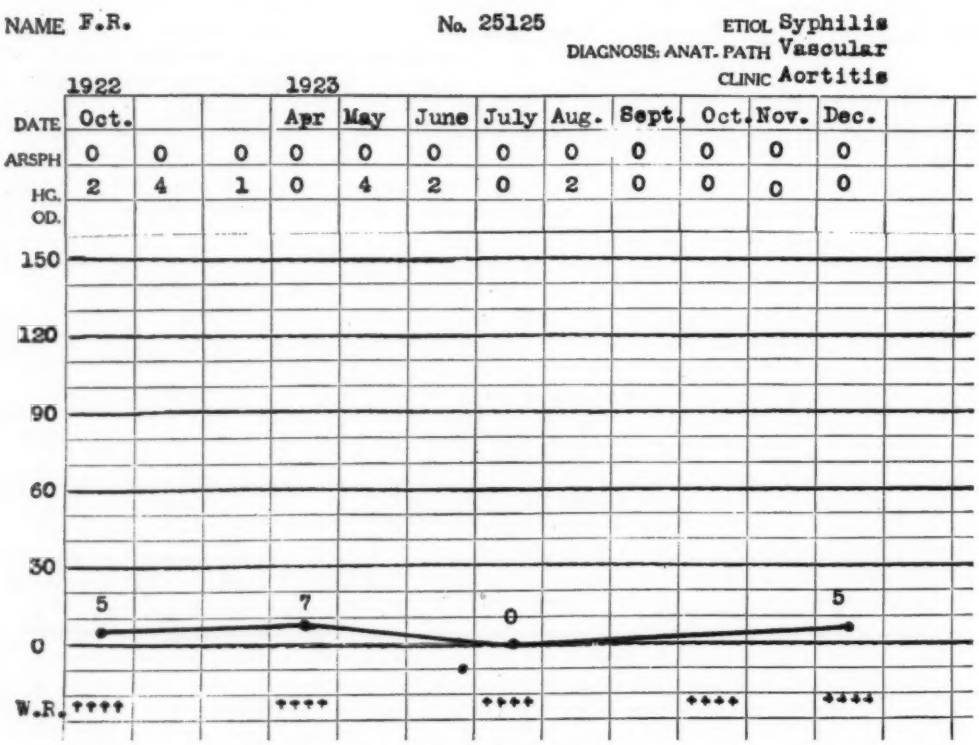

Chart XI.

tests. None of the results of the fluid examinations from nonsyphilitic cases or from any cases since the date mentioned have been submitted to a critical analysis.

For clinical purposes the cases were classified as follows:

	Fluids	Cases
Mesodermal (meningovascular) neurosyphilis	57	25
Ectodermal (parenchymatous) neurosyphilis	112	37
	169	62

The technic was that originally recommended by Vernes. A result above 50 warrants the unqualified diagnosis of neurosyphilis. Results between 30 and 50 justify a strong presumption of neurosyphilis but require more than

TABLE II

SHOWING THE SEROLOGIC RESULTS AND THE CLINICAL DIAGNOSES IN 19 BLOOD SERUMS FROM A GROUP OF CASES IN WHICH THE DIAGNOSIS OF SYPHILIS COULD NOT BE AFFIRMED CLINICALLY

NO.	W. R.	V. R.	DIAGNOSIS AND REMARKS
1.	–	5	Neurosyphilis
2.	–	3	Neurosyphilis
3.	–	0	Neurosyphilis—Rt. hemiparesis
4.	–	29	Vertigo—Syphilis denied
5.	–	76	Hemiparesis of sudden onset—Syphilis denied
6.	–	24	Psychoneurosis—Syphilis denied
7.	–	19	Wife of a paretic. No symptoms of neurosyphilis
8.	–	15	Psychoneurosis. Affirms syphilitic infection in 1918
9.	++++	7	Psychoneurosis. Syphilis admitted
10.	++++	8	Wife of a syphilitic—No symptoms
11.	++++	69	Wife of a syphilitic—No symptoms
12.	+	0	Arteriosclerosis and hypertension—Syphilis denied
13.	++++	0	Arteriosclerosis and hypertension—Syphilis denied
14.	++++	1	Right facial paresis—Syphilis admitted
15.	++++	66	Myocarditis—Syphilis denied
16.	+	7	Paranoid trend—Syphilis denied
17.	++	5	Psychoneurosis—Syphilis denied
18.	++	13	Epilepsy—Syphilis denied
19.	++++	5	Psychoneurosis—Syphilis denied

one examination in order to establish two points for the graph. The oscillation then determines the interpretation.

To utilize the analogy previously mentioned, we may repeat that as the normal temperature is subject to individual variation so is the normal flocculence of the spinal fluid. Zero may be the normal flocculence for some individuals, 10 for others and 30 for others. Normal flocculence does not exceed 50 and oscillation of more than a few points from one time to another does not occur in nonsyphilitic fluids.

On the basis of single fluid examinations the Wassermann and Vernes reactions agreed 113 times and disagreed 56 times. Of the fifty-six disagreements the Wassermann was positive and the Vernes less than 30 in 52 cases, the Wassermann was negative and Vernes more than 30 in 4.

The 56 fluids that showed a variance between the two tests in individual examinations came from 29 cases. Fifty-one other fluid examinations from these same cases were in agreement.

Inasmuch as the interpretation of this reaction depends upon a study of the curve that is obtained by plotting the results of successive examinations, we can only gain a proper idea of its value as compared with other tests by studying these curves.

An analysis of the 29 cases in which instances of variation occurred in certain single examinations disclosed the following facts:

Ten cases had but one spinal fluid examination. The Wassermann was negative and the Vernes greater than 30 in 1 case. The Wassermann was positive and the Vernes less than 30, nine times.

Three cases had two fluid examinations each. In two of these the Vernes curve agreed with Wassermann results and in the third, a case of treated tabes dorsalis, the Wassermann was positive and the Vernes less than 30.

Sixteen cases had from 3 to 7 fluid examinations each. The Vernes curves agreed with the Wassermann results in fifteen and in the sixteenth the Wassermann was negative and the Vernes curve above 30. In other words the Vernes curves indicated syphilis in all of these sixteen, the Wassermann was positive in fifteen. The case in which the Wassermann and Vernes disagreed was one of treated tabes dorsalis. The Wassermann was negative on three examinations but the Vernes showed hyperflocculence twice and normal once.

These fluid results on cases of confirmed neurosyphilis may be summarized by saying that:

1. Where three or more examinations were made, the Vernes curves indicated the activity of syphilis in sixteen cases and the Wassermann was positive in fifteen.

2. In three cases where only two examinations were made the Wassermann was positive in all and the Vernes curves gave a presumption of syphilis in two.

3. When the results of single examinations were compared (10 cases), the Wassermann was positive and the Vernes less than 30 in nine, the Wassermann was negative and the Vernes more than 30 in one.

The Vernes reaction is a valuable addition to the serologic procedures for the diagnosis of syphilis.

It is especially useful for the determination of the effect of therapy.

Successive results illustrate graphically whether the infection is progressing, remaining stationary, or regressing.

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LABORATORY METHODS

SIMPLE APPARATUS FOR CORONARY PERFUSION OF THE MAMMALIAN HEART*

By W. T. Dawson, B.A., (Oxon.), M.A., Philadelphia, Pa.

THIS apparatus is a modification of that of Gunn.[1] The principal difference lies in a more convenient arrangement for heating the perfusion fluid, by transfer of heat from a Bunsen flame by conduction along a metal rod to the water of the outer jacket, setting up convection currents in the water there, resulting in even heating of the perfusion fluid flowing along the inner tube. Such an arrangement appears to have been first used by Smith[2] and was last year independently worked out by myself, and is here reported, with the consent and by the advice of Dr. Smith, in detail, in the hope that such a description may be of value to teachers.

DESCRIPTION OF THE APPARATUS

Two Ringer-Locke reservoirs are provided, in which the Ringer is at room temperature. They may conveniently be placed 20 to 50 cm. above the top of the inner tube. They are connected through a small *Y*-tube with the inner tube of the perfusion apparatus. The upper end of the inner tube is furnished with a one-hole stopper; the tube may be of thin glass to facilitate conduction of heat but I have not found this necessary; as to diameter, it is selected of such a size as just to admit a "twelve-inch chemical" thermometer; the thermometer rests on a constriction in the wall made by denting in the latter while hot and it is thus prevented from slipping down; the constriction is about 2 cm. above a cannula end which, for the rabbit heart, is about 5 mm. outside diameter; for cats a larger and for guinea pigs and rats a smaller cannula is rather desirable. The inner tube may be cut off below the constriction so that such cannulas may be inserted in the inner tube as in Gunn's apparatus; the space between the stopper at the top and the upper end of the thermometer is filled with glass beads. The inner tube is about 38 cm. long. The outer jacket, which contains water, is about 30 cm. long and 4 cm. in diameter, provided with two side tubes, one, bulbed at its junction, for the entrance of a piece of copper wire of about 6 mm. diameter (No. 4 gauge) and 20 cm. long; the other fitted with rubber tubing and pinchcock so as to facilitate letting all or part of the warm water out of the outer jacket in order to cause a more rapid fall of temperature of the perfusion fluid in studying the effect of temperature upon rate. The size of the rod limits the amount of heat conducted into the outer jacket per unit of time;

*From Woman's Medical College, Philadelphia, Pa.
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larger sizes, such as No. 3 or No. 2 may be necessary in perfusions of hearts of larger animals such as the dog; if they can be obtained they should, therefore, be used in making up the apparatus. The connection with the side-tube of the outer jacket is made by a sleeve of rubber tubing wired in place with soft copper wire; this connection does not seem to perish very quickly, and is cheap and easily renewed. A lighted Bunsen is set under the rod a few minutes before it is desired to use the apparatus, and while the perfusion is proceeding the temperature may be readily regulated by shifting

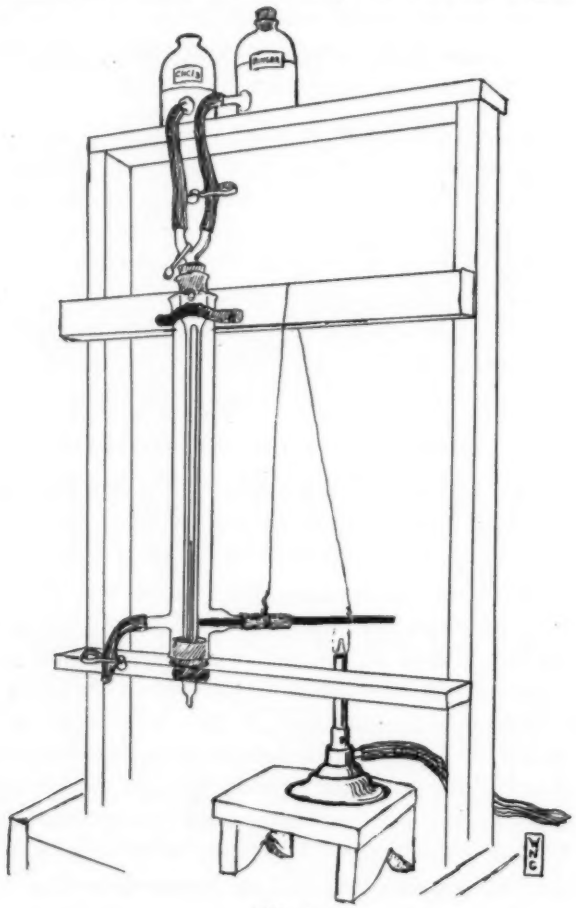

Fig. 1.

the burner to a suitable position to keep the temperature of the perfusion fluid constant. A short length of narrow rubber tubing, not shown in the diagram, is kept on the cannula end as a guard tube when the apparatus is not in use and also to aid in filling the inner tube. When this is desired the guard tube is closed off with a pinchcock, the rubber stopper at the upper end loosened and Ringer allowed to flow in from one of the reservoirs. When the inner tube is filled the stopper is pushed down into position, and a little Ringer run down through the inner tube by releasing the pinchcock on the guard tube. If both Ringer reservoirs be now clamped off, the Ringer will not escape from the inner tube when the guard tube is removed from the

cannula end to allow the heart to be tied on. The reservoirs and the inner tube should be drained of Ringer at the end of each day's work and rinsed with distilled water.

THE PERFUSION FLUID

The Ringer-Locke used has the following composition: NaCl, 0.92 per cent; KCl, 0.042 per cent; $CaCl_2$, anhydrous, 0.012 per cent; $NaHCO_3$, 0.015 per cent; several liters of this are made up in a large bottle and oxygenated by the passage through it of a stream of bubbles of oxygen for about half an hour; if well stoppered it keeps its oxygen content apparently sufficiently high for several days; just before placing it in the reservoirs one gram of glucose is added to each liter which dissolves rapidly. The formula given above agrees in calcium content with those given in the laboratory manuals of Sollman[3] and Jackson,[4] and others, assuming that "calcium chloride crystals" means $CaCl_2.6H_2O$. There are, however, several other crystalline forms. *The United States Pharmacopeia,* Edition 9, requires for general medicinal uses a calcium chloride which apparently must have the formula $CaCl_2.2H_2O$ or contain still less water than corresponds to this formula. Such a calcium chloride is, therefore, on the market. The difficulty of assigning any clear meaning to the description "calcium chloride crystals" may be easily seen from the following quotation from J. W. Mellor's *Modern Inorganic Chemistry:* "At 29.8° the hexahydrate $CaCl_2.6H_2O$ changes into the α-tetrahydrate α-$CaCl_2.4H_2O$; at 45.3° the α-tetrahydrate passes into the dihydrate $CaCl_2.2H_2O$; at 175.5° the dihydrate passes into the monohydrate $CaCl_2.H_2O$; and at 260° the monohydrate passes into the anhydrous salt $CaCl_2$." If it be true, as is very generally taught, that a delicate balance of inorganic positive ions is of supreme importance in physiologic salts solutions, the exact sort of calcium chloride used should be more exactly specified in textbooks and other publications than is now usually the case. Calcium chloride exists in at least five different forms; and each of these at ordinary temperatures and humidities is avid of water and, therefore, liable to change of calcium percentage.

PREPARATION OF THE HEART

The success of a perfusion depends largely on the condition of the heart before removal from the animal, so that it is desirable, if possible, to use hearts from freshly-killed unanesthetized animals. With bleeding, moreover, there appears to be some danger of air embolism of the coronaries. Speed is also an essential in getting the heart out of the rabbit. The animal is seized by the small of the back with the left hand; and while it hangs head down it is struck with the side of the right hand a sudden hard blow on the occiput so as to stun it completely. An assistant grasps the legs and stretches the rabbit out upon a table. With a pair of stout scissors the skin is split up the middle on the underside, and an opening at once made into the thoracic cavity by cutting through the ribs from diaphragm to neck on the right of the sternum; while this cut is being made the animal is turned on its left side so that as the lungs collapse the heart will fall away from the points of the scissors. The fingers of the two hands are now passed around

the chest of the rabbit just under the axillae, while the thumbs enter the opened thorax under the ribs at the borders of the cleft made into it. The thumbs are forced apart so as to dislocate the costal walls, which are pushed far back till the heart lies in easy sight and reach. The pericardium is picked up between thumb and forefinger, and split well open with small scissors. The heart is popped out by gentle manipulation around it, the tips of the fingers placed under it, the great vascular trunks lightly grasped with thumb and forefinger, and the heart, so protected from injury, liberated, by a semicircular cut about two centimeters from its base, from the rest of the body. The mass is thrown into a beaker of Ringer, and when this becomes much stained with blood, into a second, and so on. The heart is prepared for tying onto the perfusion cannula by now trimming off the mass cut away with it, the aorta being identified as a white stout elastic tube and cut short before the origin of the innominate artery.

With small forceps the aorta is pinched off 2 mm. below the top, its lumen filled with Ringer from a medicine dropper, and the great artery pushed up over the cannula end of the inner tube, and tied in position. The beat may start up in such a fresh heart on perfusion at once; in fact, it usually continues beating during its passage through the beakers of Ringer at room temperature; as the perfusion fluid is warmed up the rate increases (see the beautiful records of effect of temperature changes in Sherrington,[5] pp. 11, 12). The optimum temperature is about 35° to 37° C. (Dixon,[6] p. 40). The heart may be started off, if the beat is not satisfactory, by injecting with a hypodermic syringe into the rubber exit tube of the Ringer reservoir in use 1 c.c. of 1:50,000 epinephrin or of 1:5,000 epinine.

RESISTANCE OF THE HEART TO LACK OF OXYGEN

If the room temperature be 20° C. or over the heart usually continues beating in the beakers of Ringer, and is then exposed to lack of oxygen. To this it is not particularly sensitive, apparently. It has recently been found that clamping off the circulation through the heart of the cat for eight minutes did not prevent the resumption at the end of that time of vigorous, normal contraction[7]; the much more sensitive cerebral cells were damaged beyond repair by three minutes cessation of the circulation. Gunn says,[1] moreover, that excised hearts may be successfully perfused after a period of several hours lying in Ringer at room temperature. This is possible probably where the temperature is so low that the hearts practically or altogether cease to beat. I was unable to confirm it with two guinea pig hearts kept for six hours at about 20° C.; the beat was revived but was not at any time good, though improved by injection into the rubber connection tube of adrenalin and 1 per cent $CaCl_2$ solutions. Starling and Verney apparently consider that, like skeletal muscle, cardiac muscle needs oxygen only for the recovery process, the actual contraction being anerobic.[8] This appears to be confirmed by the results of Hilton and Eichholtz.[9]

My thanks are due to Prof. H. C. Bazett, of the Medical Department of the University of Pennsylvania, for his advice and for giving me the facilities for having the apparatus made up; to J. H. Graham, the university glass-

blower, for making two models; and to William N. Goodell for kindly making the drawing.

SUMMARY

A modification of Gunn's apparatus for the perfusion of the coronary vessels of the mammalian heart is described, dimensions given, and some remarks made as to its use.

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AN IMPROVED APPARATUS FOR DETERMINATION OF CHOLESTEROL*

By S. L. Leiboff, Ph.B., New York.

DURING the last decade a great deal of work has been done on the cholesterol content of the blood during health and disease and valuable information was thus obtained. We now know that increased amounts are found in chronic and acute nephritis, diabetes, toxemias of pregnancy, cholelithiasis and lipemia. A marked decrease is found in pernicious anemia.

For this knowledge we are greatly indebted to Grigaut[1] who was the first to describe a colorimetric method for determining cholesterol in small amounts of blood.

At present there are three methods in use which give fairly good results. They are the following:

1. Digitonin gravimetric method of Windaus,[2] by which the cholesterol is precipitated as a compound of digitonin, and weighed. Only free cholesterol is precipitated by the digitonin. For the estimation of the total cholesterol, the cholesterol esters must first be saponified.

2. Bloor's[3] modification of the Autenrieth and Funk[4] method. This is based on the Liebermann-Burchard test, and is carried out as follows: 3 c.c. of blood are extracted with a mixture of alcohol and ether and the extract is

*From the Biochemical Department of Lebanon Hospital Laboratory, New York.
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evaporated to dryness. The dry residue is then extracted with chloroform to which is added acetic anhydride and concentrated sulphuric acid, and left in a dark place until the color develops. It is then compared in a colorimeter with a standard containing a known amount of cholesterol.

3. Myers and Wardell method[5] which, like the former, is based on the Liebermann-Burchard test, but requires smaller amounts of blood. In this method 1 c.c. of blood is intimately mixed with plaster of Paris and dried in an electric oven. The dried plaster of Paris is then transferred to a paper extraction shell and is extracted with chloroform under a reflux condenser. Acetic anhydride and concentrated sulphuric acid are then added, and, when the color is developed, compared in a colorimeter.

Of the three methods just described, the digitonin method is the most accurate. However, it is not suitable for clinical work because it is extremely laborious and requires much skilful manipulation.

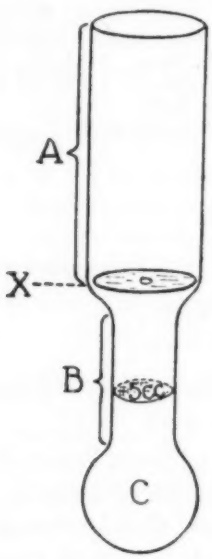

FIG. 1

FIG. 2.

Bloor's method gives somewhat higher results than the Myers-Wardell method and is considered more accurate. However, it is very tedious and requires particular attention while the alcohol-ether extract is being dried, since the slightest overheating produces a brownish substance which greatly interferes with the color formation.

Up to the present the Myers-Wardell method has been the most suitable for clinical purposes, and the most widely used. This method, while the best at our disposal, has the following disadvantages: first, it is very time-consuming, and second, it is supposed to give somewhat low figures. The latter is apparently due to slight losses incurred while removing the dried plaster of Paris to the extraction shell, and while transferring the chloroform extract from the comparatively large flask to the small test tube.

Recently, I have described a simplified method for cholesterol determination[6] which gives somewhat more accurate results and saves a great deal of

time and labor. It is carried out as follows: About 5 c.c. of chloroform are put into the extraction tube (Fig. 1) and the filter paper disc (Fig. 2) is dropped into the extraction tube; it should lie at point *X*. The constricted portion of the tube, *B*, thus serves a double purpose: it holds the paper disc in place, and permits more accurate dilution.

Twenty-five hundredths c.c. of oxalated blood is pipetted on the filter paper disc. The blood is immediately absorbed by the paper and no drying

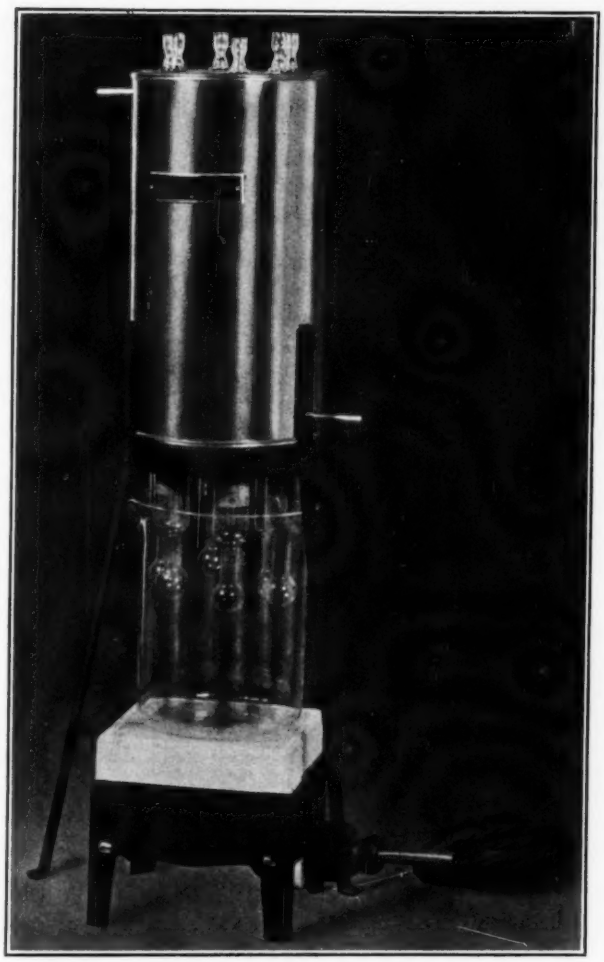

Fig. 3.

is necessary. The extraction tube is then attached to a reflux condenser and immersed in a beaker of hot or boiling water placed over a small electric stove. The water in the beaker should be above the level of the chloroform in the tube.

Extract for thirty minutes. Detach the tube from the condenser, remove the paper disc from the tube, and cool by immersing in cold water for a minute. When cool, add chloroform exactly to the 5 c.c. mark.

In a similar extraction tube place 5 c.c. of a standard solution containing

0.4 mg. of cholosterol per 5 c.c. This standard is prepared by dissolving 40 mg. of pure dry cholesterol in 500 c.c. of pure dry chloroform.

Add 2 c.c. of pure dry acetic anhydride and 0.1 c.c. of concentrated sulphuric acid to each tube. Insert cork stoppers into the tubes and invert twice to mix well. Place the tubes in a beaker of cold water for half a minute to cool, and leave in a dark place for ten minutes and read in colorimeter.

The standard is set in the colorimeter at 10 or 15 mm., depending on the intensity of the color of the unknown. The results are calculated by the following simple formula:

$$\frac{S}{R} \times 160 = \text{mg. per 100 c.c. of blood.}$$

S = reading of standard.
R = reading of unknown.

This method was checked up by the Myers-Wardell method. Cholesterol determinations were done by the two methods on blood specimens from dif-

TABLE I

SAMPLE NO.	M. AND W. METHOD MG. PER 100 C.C.	NEW METHOD MG. PER 100 C.C.	DIFFERENCE
1	165	174	+ 9
2	127	135	+ 6
3	220	233	+13
4	153	150	− 3
5	148	153	+ 5
6	139	146	+ 7
7	245	260	+15
8	129	134	+ 5
9	174	179	+ 5
10	140	137	− 3
11	165	172	+ 7
12	210	220	+10
13	143	150	+ 7
14	150	158	+ 8
15	154	165	+11
16	140	142	+ 2
17	158	170	+12
18	250	264	+14
19	160	155	− 5
20	140	140	0
21	145	152	+ 7
22	128	132	+ 4
23	139	145	+ 6
24	148	155	+ 7
25	180	190	+10
26	160	165	+ 5
27	190	185	− 5
28	154	163	+ 9
29	135	142	+ 7
30	205	223	+18
31	160	154	− 6
32	150	150	0
33	142	150	+ 8
34	180	190	+10
35	135	140	+ 5
36	270	290	+20
37	155	160	+ 5
38	170	180	+10
39	310	325	+15
40	145	150	+ 5

ferent patients. The blood specimens were those sent to the laboratory for routine blood chemistry.

Table I gives the result of cholesterol determinations on forty specimens of blood.

As is shown in Table I, the new method gave slightly higher results than the Myers-Wardell method. This was to be expected, since the slightly lower results obtained with the Myers-Wardell method are due, as I pointed out before, to the loss caused by the transfer of material. Since in the new method no transfers of material are made at any time, no loss is possible.

To further facilitate the work of cholesterol determinations I have devised a special multiple condenser (Fig. 3) which allows six simultaneous extractions. The condenser is made of copper. Its diameter is 5 inches and its length 9 inches. It is held firmly by an iron tripod. Through the condenser pass six glass tubes. These tubes have a diminished diameter at each end. The lower end of each tube passes through a cork stopper which fits into the extraction tube. The upper ends are funnel-shaped through which may be added chloroform while in operation, if necessary. The glass tubes could easily be removed and replaced by new ones in case of breakage. The condenser is absolutely leak-proof.

The filter paper discs are especially prepared from very thick fat-free filter paper. They have a diameter of $\frac{3}{4}$ inch and a thickness of $\frac{1}{16}$ inch. The discs are perforated by a small hole so as to allow the escape of vapor into the condenser.

I wish to thank Samuel Gittlow and Milton J. Goodfriend, of the Lebanon Hospital, for their valuable suggestions and criticism, and for their checking up the accuracy of the method.

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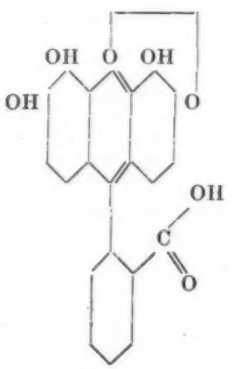
URAN-GALLEIN, A NEW RAPID ELASTIC TISSUE STAIN PRODUCING SIMULTANEOUS STAINING OF NUCLEI AND PROTOPLASM*

By Frederick Proescher, M.D., and Albert Paul Krueger, A.B.,
Agnew, California

FOR the selective staining of elastic tissue, we have two excellent methods, namely, that of Taenzer-Unna and that of Weigert. Both methods are absolutely dependable and stain the finest ramifications of the elastic elements, but possess in common the disadvantage of being too cumbersome for quick orientation.

In the course of staining experiments with gallein, we found that the latter in combination with uranium produced a mordant dye which is readily prepared and stains elastin in a few minutes with simultaneous staining of nuclei and protoplasm.

Gallein is dioxyfluorescein and is obtained by condensation of 1, 2, 3, trioxybenzol (pyrogallol) with phthalic acid anhydride (Bayer). It contains two OH groups in close relation to the chromophoric oxygen atoms, this molecular arrangement making possible the formation of a mordant dye with a metallic salt.



Gallein is not unknown to histologic technic but is used very little. In combination with aluminum sulphate or aluminum chloride, it is recommended as a good nuclear stain. It is marketed in the form of a violet paste or powder. It is practically insoluble in cold water, ether, benzol, or chloroform and dissolves in alcohol producing a dark violet color. With NaOH the solution turns blue and when treated with HCl develops a brown color. The dye is soluble in H_2SO_4forming a red solution. Gallein treated with chromium or iron salts yields a beautiful violet-red mordant. The mordant prepared by treatment with aluminum sulphate has more of a purple tinge. If an excess of gallein is added to 2 per cent or 3 per cent alum solution or better a similar

*From the Pathological Laboratory, Agnew State Hospital, Agnew, California.
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solution of aluminum sulphate or chloride and the mixture is boiled for a few minutes a stain is produced which stains nuclei very distinctly.

PREPARATION OF THE STAINING SOLUTION

One and five-tenths gm. uranium acetate, 2.0 gm. sodium citrate, and 0.2 gm. sodium carbonate are dissolved in 50 c.c. distilled water. This solution will keep for three weeks or a month if placed in tightly stoppered bottles. It may be kept indefinitely if sterilized and placed in the dark. Light causes it to turn brown but does not in any way render it unfit for use. One-tenth gm. gallein (Merck) is added to 10 c.c. of the uranium solution, the mixture is boiled for two minutes and the solution cooled. The resultant purplish red solution will keep three or four weeks, if kept in the dark, and if desired may be filtered before use.

STAINING METHOD

The tissue should be fixed in formalin or better Muller-formol. For rapid work we prefer frozen sections from formalin or Muller-formol fixed tissue. However, embedding in paraffin or celloidin does not interfere with the staining.

The sections are immersed in the staining solution for five to ten minutes and washed with water (tap or distilled) for one or two minutes. If very fine differentiation is desired the sections should be washed in water for five to ten minutes. Prolonged washing does not alter the degree of elastic tissue staining but slowly decolorizes both protoplasms and nuclei.

For quick orientation the sections may be mounted in glycerin. They will keep for several days in that medium but slowly decolorize. For permanent mounting the sections, after washing in water, are dehydrated in absolute alcohol for three to four minutes, cleared in bergamot oil, and mounted in dammar balsam.

Counter staining is not necessary since nuclei and protoplasm are well differentiated. Nuclei are stained purplish red, protoplasm lavender, red cells deep brownish red and elastic fibers deep violet blue. Connective tissue appears light brownish red, muscle tissue deep brownish red to purplish red.

Immersion of the sections for one-half to one minute in a 0.25 per cent aqueous picric acid solution produces a yellowish brown to yellowish red differentiation of nuclei and protoplasm. After such treatment elastic fibers appear dark blue to blue black. If the sections are mounted in glycerin the picric acid treatment acts as a fixative preventing decolorization in the mounting medium.

PRESERVATION OF ANTISHEEP AMBOCEPTOR WITH GLYCERIN*

By Virginia Langworthy, M.S., and Margaret Wemple, A.B., Albany, N. Y.

THE necessity for maintaining a supply of antisheep amboceptor sufficient for performing a large number of complement-fixation tests as well as for distribution to local laboratories throughout New York State led to a trial, in 1920, of the Clock and Beard[1] method of preserving amboceptor with 50 per cent of glycerin. It had been noted that in amboceptor sterilized according to the method previously followed, that is, heating for thirty minutes at 54° C. on three successive days, there is usually a decrease in titer after nine to twelve months' storage and it frequently becomes contaminated during use in spite of the attempt to observe strict aseptic precautions when withdrawing portions from the stock supply. The results of our four years' experience with the use of glycerin as a preservative for hemolytic serum have been so favorable that it seems important to add another endorsement to those which have already been published.[2, 3]

Comparative tests have been made with sterilized and glycerinated portions of hemolytic serums from ten different rabbits to determine the following points: (1) the effectiveness of glycerin in preventing the growth of common contaminants, (2) the rate of deterioration in the hemolytic titer of glycerinated amboceptor as compared with sterilized amboceptor, and (3) the effect of glycerin upon the complement-fixation reaction in syphilis.

EFFECTIVENESS OF GLYCERIN IN PREVENTING GROWTH OF BACTERIA AND MOULDS

Glycerinated portions of each of three amboceptors inoculated with B. subtilis, with a staphylococcus and a common mould showed no growth after four weeks at room temperature, while sterilized portions inoculated with the same cultures showed a heavy growth after five to six days at room temperature. The fact that glycerin prevents the growth of common contaminants, which was thus experimentally demonstrated, has since been fully confirmed by the use of glycerinated amboceptor for routine work, during three years.

COMPARISON OF HEMOLYTIC TITER OF THE STERILIZED AND GLYCERINATED AMBOCEPTORS AT THE END OF THE STORAGE PERIODS

In testing the hemolytic titer at intervals during storage, the titrations of both the sterilized and the glycerinated portions of each amboceptor were made on the same day, and with the same complement and the same suspension of sheep cells.

After four years' storage at 3 to 6° C., nine of the ten glycerinated serums showed no change in titer. The other serum preserved with glycerin showed a slight decrease in titer at the end of eighteen months. Of the ten sterilized serums, only four showed no change in titer at the end of four years. Five serums showed a slight decrease in titer, one at the end of nine months, three at the end of three years, and one at the end of four years; one showed a marked decrease in titer at the end of four years. It might be added in this connection that a large supply of antisheep amboceptor produced in a mule, in

*From the Division of Laboratories and Research, New York State Department of Health, Albany, N. Y. Augustus Wadsworth, M.D., Director.
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1921, was preserved with glycerin at that time and has shown practically no change in titer up to the present.

Not only did the glycerinated portions show less deterioration in titer, but they contained less precipitate than the sterilized portions, after long periods of storage. No constant relation was observed, however, between the formation of precipitate and loss of hemolytic activity, as two of the sterilized serums which showed no decrease in titer contained a large amount of precipitate.

EFFECT OF GLYCERIN UPON THE COMPLEMENT-FIXATION REACTION

In a large series of complement-fixation tests, cells sensitized with glycerinated amboceptor of 1:1000 titer† gave results agreeing very closely with those of routine tests in which cells were used which had been sensitized with amboceptor containing no glycerin. The few differences which did occur were slight and within the limits of technical inaccuracies.

Since a high concentration of glycerin would result in the test if 50 per cent of glycerin were added to an amboceptor of low titer, experiments were made to determine the maximum amount of glycerin which could be present without influencing the complement-fixation reaction. When the concentration of glycerin in the diluted amboceptor was as high as 1:10, slightly more complement was required for hemolysis, and when the concentration was from 1:10 to 1:50, some lysis of the sensitized cells was evident if they stood for two to three hours at room temperature. The use of amboceptor containing these concentrations of glycerin did not affect the complement-fixation reactions, however, when the appropriate amount of complement was used and the cells were added to tests fifteen minutes after sensitization. When the concentration of glycerin was only 1:100 or less, there was no appreciable effect upon the cells or upon the results of complement-fixation tests. Since an amboceptor of titer lower than 1:100 would not ordinarily be used, the effect of the amount of glycerin introduced through amboceptor preserved with 50 per cent of glycerin appears to be negligible.

CONCLUSION

The results of these experiments have indicated that the addition of 50 per cent of glycerin is a practical and efficient method of preserving amboceptor. It is effective in preventing the growth in serum of common contaminants; the rate of deterioration in the hemolytic titer of glycerinated amboceptor is, in general, less than that of sterilized amboceptor, the titer of the former remaining unchanged for a period of at least four years; and 50 per cent of glycerin added to a hemolytic serum of reasonably high titer has no influence upon the complement-fixation reaction in syphilis.

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†The titer is expressed in terms of the dilution which contains two units in 0.1 c.c. Since 0.1 c.c. of diluted amboceptor is used in a test having a total volume of 0.5 c.c., the final concentration of glycerin in the test is only one-fifth as great, e.g., in this instance, 1:5000.

A NEW BACTERIOLOGIC STAIN*

By L. E. Harrison, Prescott, Arizona

THE stain to be described was developed following the observation that formalin added to an aqueous solution of basic fuchsin containing phenol, increased its fastness for bacteria, and after standing for two weeks, changed from deep red to a dark purple. After some experimentation, the writer worked out the following formula:

One gram of basic fuchsin is added to 100 c.c of distilled water and gently heated to 100° C. The solution is then filtered. To 75 c.c. of filtrate, 10 c.c. of 37 per cent formaldehyde, 10 c.c. of saturated aqueous solution of phenol, and 5 c.c. of glycerol are added. The stain is now placed in a glass stoppered bottle and allowed to stand for two weeks. At the end of that time it appears that polymerization of the fuchsin in the presence of formaldehyde has taken place with the production of a dark purple stain. The stain seems to become darker on standing, and improves in staining quality. Deterioration does not take place.

For staining tubercle bacilli, a few drops of the stain are added to the smear and heated over a flame until steaming. The slide is then set aside for about four minutes. At the end of this time the smear is decolorized with an alcoholic sulphuric acid solution (15 parts of concentrated sulphuric acid to 85 parts of 95 per cent of ethyl alcohol). The smear is now washed with tap water and counterstained for two minutes with a saturated alcoholic solution of picric acid to which has been added 5 c.c. of Lugol's solution for each 95 c.c.

A NEW STAIN

The tubercle bacilli stain with remarkable clearness as brownish black rod-like forms against a light brownish yellow background. Beaded forms are particularly prominent.

The organisms of Vincent's angina are readily stained with this method. The smear is stained in the same manner as for staining the tubercle bacilli. It is then decolorized with 95 per cent alcohol, and counterstained with the picric acid iodine solution. The spirochetes stand out as dark spirals against a yellow background.

Other organisms may be stained with this method. Gram-positive bacteria are most suitable. The diphtheroid group seem to be especially well stained. Spore forms are also well stained.

*From The Pamsetgaaf Laboratory, Prescott, Arizona.
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A NOTE ON THE USE OF THE SWIMMING CUP FOR WASHING TISSUES*

By Elizabeth Morford, B.A., Houston, Texas.

FOR efficiency in washing tissue free of fixing solution, float it in a swimming cup. This is a porcelain cup or thimble with perforations in the sides and fitted with a cork stopper which floats the cup in the washing fluid.

If the cup is set to wash in a half liter beaker under a slowly running faucet, the current produced in the water is strong enough to keep the cup jostling and bobbing and the holes in the cup are large enough to allow the water to run through the cup freely.

There is no danger of the piece of tissue being lost and there is the assurance that it is constantly being bathed in fresh water. The cup in the large size nicely accommodates several blocks of tissue that have been sampled from the gross block.

The swimming cup was planned originally for washing specimens of ore. It will be found illustrated in the Arthur Thomas catalogue.

Communication

In the January issue of the Journal of Laboratory and Clinical Medicine, there appeared an article by C. E. Reynar, entitled, "The Red Corpuscle Suspension for the Kolmer-Wassermann Reaction."

We wish to draw attention to the fact that we published an article entitled "The Use of Formalinized Sheep Cells in Complement-Fixation Tests" which appeared in the Zeitschrift für Immunitätsforschung und Experimentelle Therapie for June 1912.

In this article we covered the subject of Dr. Reynar's work completely, arriving at similar conclusions.

Since that time our article has been quoted a number of times and the method of preserving sheep cells for complement-fixation tests originated by us has been in use in our own and in other laboratories.

We are greatly surprised that Dr. Reynar overlooked our article and we wish to take this opportunity of drawing the attention of your readers to our work which antedated Reynar's by so many years.

Yours very truly,

(Signed) Bernstein and Koliski.

New York, May 19, 1925.

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EDITORIALS

Cervical Sympathectomy in Angina Pectoris

MOST people first consult their physician for the relief of pain or discomfort. Their primary interest is in obtaining such relief and he who can accomplish this most readily and satisfactorily is considered the best physician. Nondescript pain will usually respond to routine therapeutic measures and often the physician relieves the pain before he has had an opportunity to study it, to learn of its origin or mode of action. Volumes have been written on pain alone and yet our knowledge of this symptom is still quite superficial. The experimental difficulties are obvious. A person suffering pain of any degree will not consent long to a scientific study while feeling that proper therapeutic measures would give prompt relief. The experimental animal can only describe his pain in a general way. Besides, the principles of humaneness deter us from inflicting any considerable unnecessary pain.

The victim of angina obviously desires, foremost, relief from the excruciating pain. Relief at best is usually temporary. It is, therefore, not surprising

that the work of Jonnesco, first reported in 1916, and revived by Brown and Coffey, in 1923, has received considerable interested study and the enthusiastic support of many. Cervical sympathectomy usually relieves the pain of angina, often permanently.

Various writers, particularly Sir James MacKenzie, whose authority it would be presumptuous to ignore, have questioned the advisability of removing a danger signal whose absence may result direly. Pain notifies when the heart is being overworked and forces the victim to stop all exertion. It is in part at least a protective measure.

Morphine is one of the most valuable drugs for relief of pain. It is often clearly indicated and without doubt has been life saving; yet we would display the poorest sort of judgment were we to use morphine for all pains. Likewise in angina, cervical sympathectomy relieves pain. It appears probable that in certain types of angina or in angina attributable to certain causes, the operation will be found to have a legitimate therapeutic place. The work is too recent for a clear-cut statement of operative indications, but since it is apparently coming into rather general use, since it is readily agreed to by the patient, and since it opens a new field for surgical hyperenthusiasm, it is well to study the work done so far, in an attempt to find some broad general lines to follow.

The first observation after operation and naturally the most striking is the immediate and usually complete relief from pain. The second observation gained from a study of the literature is that while the results are admirable, some patients die relatively soon after recovery from operation. They die without pain to be sure, but experiencing the familiar sensation of thoracic tightness and anxiety with fear of impending dissolution.

Generalizing, we may say that where there is evidence of extensive and grave myocardial damage the operation removes a valuable warning mechanism and predisposes to a relatively early death from unexpected overexertion. Thus, one patient about two weeks after convalescence from a successful operation, had talked with the physician making his rounds and was in excellent state. The doctor having left the room, the patient recalled a question which he wished to ask, jumped up and hurried into the hall. He suddenly experienced an intense uneasiness and anxiety, and with no pain whatsoever, promptly died. In cases with long-standing advanced myocarditis with or without auricular fibrillation or valvular disease, the advisability of operation is open to grave question. If it is performed, the patient's activities must be controlled with a degree of care and over a period of time, difficult or indeed impossible to accomplish. Where angina is the result of recurrent coronary thrombosis with infarction, grave myocardial damage must of necessity be present.

However, true angina pectoris not infrequently occurs in individuals in whom remarkably little physical or roentgenologic evidence of cardiac damage is observed. As time goes on we will probably find that the operation will be applied particularly in this group. MacKenzie, however, has stressed that such individuals have often been relieved for years by more conservative methods. The rationality of cervical sympathectomy will depend ultimately upon further

elucidation concerning the cause of angina. This will naturally lead to the classification of the disease into several different groups.

MacKenzie states that angina is still a matter for vague speculation and that the employment of such drastic treatment as an operation for a condition so little known does not redound to the credit of medicine. True, the operation is in a sense empiric but the results are definite and the same criticism might equally apply to many other successful surgical procedures. He points out that pain and other symptoms associated with angina pectoris are not infrequent in people whose hearts are healthy, but who suffer from ill health which disturbs the nervous system. The majority of these are women under fifty years of age. During the war numbers of young soldiers showed all the signs of angina pectoris but they suffered from some infection often too obscure for identification but which in some instances was ultimately found to be trench fever. He classifies these as "secondary angina." Such cases, followed by MacKenzie for many years have in no instance died from an affection of the heart. On the other hand, in the majority of cases of primary angina, morbid changes are found at necropsy in the coronary arteries and in the muscles of the left ventricle. These consist of narrowing of the lumen in some part of the coronary arteries such that the supply of blood to the muscle is sufficiently diminished to impair functional efficiency and to cause degeneration and destructive changes in the heart muscle. The healthy coronary artery is very distensible and readily accommodates great increase in the blood supply when the heart is called upon to increase its work.

Wiggers calls attention to the milking action of the heart muscle serving to increase the coronary blood supply. The heart does not beat during perfusion with an oxygen-free solution. If adrenalin be added to the solution, the flow through the coronaries is *decreased* from constriction to the peripheral vessels. On the other hand, a heart perfused with oxygenated Ringer's solution continues to beat and adrenalin this time *increases* the blood flow, since it simultaneously increases the rate and amplitude of ventricular contraction, which is sufficient to overpower the constrictor effect upon the coronary vessels themselves. Such milking action ceases to exist when the arteries become hardened by sclerosis or calcification, or obliterated by endarteritis.

While the pathologic picture in angina is usually that of coronary sclerosis, many persons come to autopsy with coronary sclerosis but who have never, as far as can be ascertained, experienced the symptoms of angina. Sclerosis without angina may exist, but true primary angina probably does not occur without some coronary involvement. Sclerosis alone, according to MacKenzie, is probably insufficient to account for angina. Nearly always some myocardial impairment accompanies the vascular change.

What causes the pain of angina? What occurs within the heart synchronous with the pain? In general, three explanations have been seriously considered. The condition may be associated with a deficient blood supply. Intermittent claudication in the peripheral muscles, likewise associated with arteriosclerosis, results from local anemia and is accompanied by intense pain. The two diseases may be quite similar.

A second theory and one which we may consider as corollary to the first, is that during the anginal attack, a spasm occurs in vessels already narrowed by sclerosis. Vasoconstriction is not a necessary part of the first hypothesis in that with coronary sclerosis the blood supply may be diminished without concomitant spasm. Vasomotor fibers have been shown to exist in the coronary vessels but it has not been demonstrated that constriction of these vessels is the cause of angina.

Sir Clifford Allbutt associated the pain with stretching of the aorta which produces pressure on the sensory end organs in the adventitia of this vessel.

Considering the more generally accepted theories, pain is admittedly associated with muscular spasm. Myocardial spasm may result from inadequate blood supply. But what produces the pain? Is it attributable, as has been suggested, to chemical stimulation of accumulated waste products in the muscle cells, or to pressure of the spastic muscle on sensory nerves? Some hold that there are no sensory nerves in the heart. Pain is then explained as a visceromotor reflex, the afferent stimuli of which so alter the irritability of those spinal cord segments from which its nerves arise that the pain is referred to the skeletal sensory nerves having their terminals in the same segments. This would explain the distribution of the pain through the chest and arm. Along with the viscerosensory reflex a visceromotor reflex is set up with resulting constriction of the intercostal muscles. According to the hypothesis, this visceromotor reflex accounts for the sense of thoracic constriction.

While coronary embolism or thrombosis usually is associated with a more lasting pain and is more often fatal during the acute attack, recurrent small thromboses have been responsible for typical recurrent angina, lasting over a relatively long period.

Coming again to cervical sympathectomy, MacKenzie has taken rather vigorous opposition to the procedure. All that the surgeon could hope to accomplish, he says, would be to cut the nerves which convey those impulses giving rise to pain, from the damaged heart to the center of consciousness. He believes that this is not for the best interest of the patient. Pain is a signal which is given out by a muscle when exhausted and particularly when forced to work with a defective blood supply. The failure to recognize pain may lead the individual to persist in efforts beyond the heart's capacity. He affirms that while the pain accompanies a condition which is dangerous, the pain in and of itself is not dangerous.

Shortly after the publication of MacKenzie's criticism, J. N. Langley presented a communication supporting MacKenzie's contention. He stated that the theory on which most of the operations have been made is that a considerable number of sensory fibers of the heart lead to the central nervous system by way of the cervical sympathetic and vertebral nerves. He describes several anatomic and physiologic studies which would show that severing of the sympathetic trunk cuts no appreciable number of afferent fibers.

Granting that few if any sensory nerves are cut during removal of the cervical sympathetic ganglia, why is it that as obviously occurs, the pain is relieved?

Ransom agrees with Langley in his observation that no sensory nerves are found in the upper portion of the sympathetic tract. He has traced sensory nerves from the heart as high as the middle cervical ganglion but from here and from the lower cervical ganglion they pass over with the cardiac branches of the vagus, to the vagus nerve and ascend with it. It appears quite clear that particularly where the superior cervical ganglion alone is removed, as in the operation recommended by Coffey and Brown, no noteworthy sensory nerves are divided. Unlike Langley, however, Ransom and Holmes do not look upon this as a reason why the operation should not be performed. Recognizing that some change must have taken place whereby the patient was relieved of his most distressing subjective symptom, they hypothecate that in the operation, efferent nerves, probably vasoconstrictor, have been divided. Wiggers has shown that stimulation of the vagus nerve causes constriction of the coronary arteries. Holmes and Ransom believe that the vasoconstrictor fibers found by Wiggers in the vagus have their origin in the superior cervical sympathetic ganglion. They quote certain embryologic data tending to support this view. While it may be that, as suggested, the beneficial results from superior cervical sympathectomy come from dividing efferent rather than afferent nerves, we have ample evidence that considerable disturbance in various sensory nerves has been effected by the operation. The pain above the left scapula described by various writers, the jaw pain at the beginning of mastication, trigeminal neuralgic symptoms, are clearly sensory symptoms. In the more extensive operation done by Reid, a few cases have developed more marked sensory changes in the left half of the body from the head to the costal margin, with absence of epicritic sensibility, lowered threshold for pressure pain and for temperature, diminished protopathic sensibility, disturbed stereognosis, etc.

Reid's suggestion, that without clinically recognizable evidence of cardiac or aortic disease angina may be analagous to trigeminal neuralgia, is worthy of consideration. In such cases removal of the cervical ganglion would correspond to removal of the semilunar ganglion in tic douloureux. Brunning has reported pathologic changes in the sympathetic ganglia of a case of angina who was relieved by operation. These changes consisted of tissue increase, thick walled vessels, perivascular and intercellular lymphocytic infiltration, and enlarged ganglion cells apparently involved in degenerative changes.

Summarizing the evidence reviewed, we may say that there is justification for operative procedure in some cases of angina, particularly those with little physical or roentgenologic evidence of degenerative disease of the heart, aorta, or peripheral vessels. Reid and Freidlander report a case with sudden death in whom the physical findings showed little evidence of cardiac pathology, but the patient had considerable pulmonary emphysema and from the location of the cardiac impulse it seems probable that fluoroscopic examination would have shown marked cardiac hypertrophy. In all probability there was considerable myocarditis in this case. In properly selected cases the results have occasionally been spectacular. Bacon's patient seventy-eight years old, was able to walk three miles at a time without resting and without discomfort. Diez describes a man forty-two years old who had suffered from angina for eleven years, with

attacks increasing so that at the time of operation he was having an average of three a day. Since the operation he has had no pain, nor dyspnea even after an eleven mile walk.

The case must be most carefully selected from the viewpoint of cardiac pathology. The internist must decide as to the advisability of surgical intervention and must follow the case most carefully thereafter. In those so far reported not sufficient attention appears to have been given to electrocardiographic studies. Thus, an individual under the personal observation of the reviewer, with characteristic true angina but with no evidence on physical or fluoroscopic examination of noteworthy myocardial, aortic, or vascular damage. showed an inversion of the T-wave in lead two, indicating distinct myocardial change. We feel that in the preoperative and postoperative study of these cases, the electrocardiograph will find a wide field of usefulness and that its application will ultimately be of help in selecting the suitable case.

While little claim has been made for the operation beyond palliative effect and indeed little further can be claimed until more is learned of the innervation of the heart, the work reviewed suggests that we may find a truly curative tendency in a small proportion of the cases. Many cases come to autopsy with sclerosis of the coronary vessels who have never experienced angina. If in addition to sclerosis, an angiospasm is requisite to the development of angina and if operation, by severing the efferent fibers prevents this spasm, we may expect most excellent results in those cases where the cardiac lesion is limited to coronary sclerosis. Such strict limitation, however, is probably not common.

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—*W. T. V.*

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State Pathological Society of Texas

The ninth annual meeting of the State Pathological Society of Texas was held in Austin, May 4, 1925.

The following papers were presented:

President's Address. By Dr. B. F. Stout, San Antonio.

Is the Pathologist Properly Appreciated? By Dr. M. E. Bledsoe, Port Arthur.

Syphilitic Aortitis with Presentation of Specimens. By Dr. Willis W. Waite, El Paso.

Cardiac Infarcts. By Dr. G. Werley, El Paso.

Basal Metabolism Determination. By Dr. J. H. Black, Dallas.

Tumors of the Nervous Tissue of the Adrenal Body. By Dr. Henry Hartman, Galveston.

Primary Carcinoma of the Liver, with Report of Four Cases. By Dr. W. H. Hill, San Antonio.

Pernicious Anemia. By Dr. J. G. Burns, Cuero.